Immunoassay Buffer

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contents

Limiting Cross Reactivity in Immunoassays

Antibodies are used in Immunoassays to easily and specifically distinguish between different substances.

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There are several types of these assays including Enzyme linked immunosorbent assays (ELISA), enzyme immunoassays (EIA), Western blotting, radioactive labelled immunoassays (RIA), protein arrays, immunohistochemistry, and immuno-polymerase-chain reaction (Immuno-PCR). Each of these assays have one drawback in common - cross reactivity.

Immunoassays are a very important tool in bioanalytical and biochemical laboratories. They are used in research, food and environmental monitoring as well as in diagnostic applications. Immunoassays are quite easy to carry out and very specific in terms of quantitative and qualitative significance due to their use of antibodies for detection. Theoretically, each antibody can identify one antigen and binds this antigen with high affinity which explains why one can distinguish so easily between different substances.

In practice, it is not that simple. Immunoassays suffer from cross reactivity which results in false bands in Western blots, signals in the negative control of an ELISA or a very high background in a protein array. Every false result means more work, additional costs and potentially misdiagnosis of patients [1]. Although antibodies are very specific and have high affinity for one antigen in particular, often antibodies can also bind with lower affinity to other antigens which are not detected by the assay. This is even observed with very

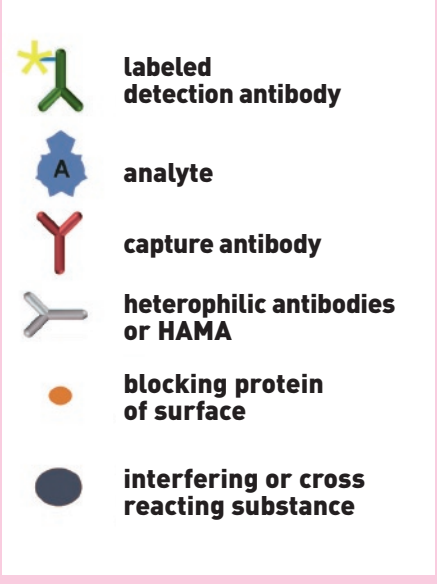

Fig. 1
The perfection: interference-free sandwich assay.

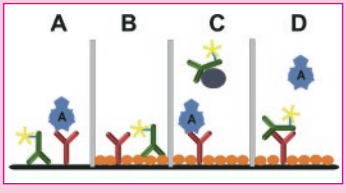

Fig. 2
A Non-specific binding of a labeled detection antibody to a not (sufficiently) blocked surface.
Result: false-positive signal.
B Non-specific binding of a labeled detection antibody to a blocked surface. Despite blocking of the surface the antibody binds to the blocking protein itself.
Result: false-positive signal.
C An interfering protein binds to the Fc segment of the detection antibody and hinders sterically the binding of the analyte.
Result: false-negative signal.
D The capture antibody binds to the Fc segment of the labeled detection antibody. The analyte cannot be bound by the capture antibody any more.
Result: false-positive signals

well characterised antibodies known to have a high affinity to the target analyte.

The result are interferences such as non-specific binding, cross reactivities and matrix effects leading to high background with bad signal-to-noise ratio.

Interferences in immunoassays

Interference can come in several forms such as cross reactivities, non-specific binding and matrix effects. Laboratory or clinical samples may contain foreign substances in concentrations that can interact with the analyte or the capture/detection antibodies thereby disrupting the desired reaction. Similarly, surfaces that act as platforms for immunoassays have also been known to be a source of interference. By applying novel buffers (e.g. CrossDown Buffer), most of the above mentioned effects can be avoided. Simply exchange the sample buffer or antibody dilution buffer by CrossDown Buffer and thereby improve the quality of the assays and the efficiency of the assay development.

To understand the basic principles of interference, we first take a look at an ideal sandwich ELISA (fig. 1) followed by fig. 2–5 where interference is demonstrated. In a trouble-free sandwich ELISA, the capture antibody is immobilised on the bottom of a well. The rest of the surface is blocked sufficiently. The capture antibody binds the analyte, while the secondary labelled antibody, binds to a different site on the analyte (fig. 1).

Non-specific binding

Non-specific binding occurs when an antibody binds to substances present in much higher concentrations than the target analyte (e.g. non-specific binding to albumin or immunoglobulins), binding to surfaces (e.g. Western blotting membranes or ELISA wells), or binding to loci on immobilized antibodies in protein arrays [2]. Assays with insufficient blocking or with difficult matrices containing e.g. a high albumin concentration or high concentration of endogenous interfering substances, are strongly affected. There are also other causes of non-specific binding.

Since detection antibodies are labelled with enzymes (e.g. alkaline Phosphatase or Peroxidase), fluorescent dyes, radioactive isotopes or DNA (Immuno-PCR), the label itself can also be a source of unwanted interactions.

In the case of fluorescent dyes, which are frequently hydrophobic, the binding properties of detection antibodies can be changed. The dyes themselves may cause unwanted binding and thereby reduce the solubility of the labelled protein. The antigen-antibody binding can be impaired too [3]. These effects may lead to increased non-specific binding of the labelled antibody onto surfaces (fig. 2A and 2B), to foreign proteins of the real sample (fig. 2C) or to the capture antibody (fig. 2D). In those cases false positive results are obtained even in the absence of an analyte or the whole assay shows a high background, respectively. In protein arrays this phenomenon leads to higher background fluorescence of single spots or a low signal-to-noise

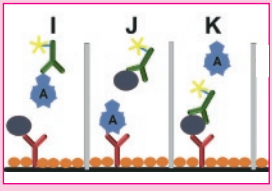

Fig. 3

I Cross reactivity of an interfering substance with the capture antibody.
Result: false-negative signal.

J Cross reactivity of an interfering substance with the detection antibody.
Result: false-negative signal.

K Cross reactivity both with the capture and with the detection antibody.
Result: false-positive signals
Such a phenomenon is rather seldom in practice, but definitely possible with antibodies having lower specificity. Such an interference picture may occur with antibodies directed to a target with a conserved amino acid sequence of a protein whose sequence motive also occurs in other proteins.

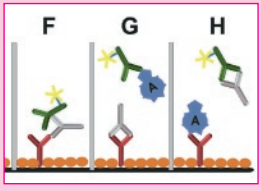

Fig. 4

F Bridging by HAMAs and heterophilic antibodies, respectively, resulting in a coupling of the capture and detection antibody.
Result: false-positive signals.

G An anti-idiotypic HAMA binding to the capture antibody. The interfering antibody binds in the area of the highly variable region of the Fab segment and thus prevents the binding of the analyte.
Result: false-negative signal.

H An anti-idiotypic HAMA binding to the detection antibody. The interfering antibody binds in the area of the highly variable region of the Fab segment and prevents the binding of the analyte.
Result: false-negative signal.

Fig. 5

L Masking of the analyte by a protein of the specimen. The epitope is blocked for binding of the capture antibody, resulting in no binding to the analyte at all or in the case of a sterical hindrance binding is very week.
Result: false-negative signal.

ratio, respectively. Fluorescent dyes also may bind proteins or antibodies from serum samples, resulting in a reduction of the dye fluorescence and in extreme cases to a complete quenching of the signal. Based on this, discussions are on-going to eliminate fluorescent dyes from protein arrays completely [4]. The complexity of protein arrays is very high due to the application of many different capture antibodies and labelled detection antibodies in one reaction. Thereby, the risk of non-specific binding of proteins in the sample or labelled antibodies to single spots increases significantly as well as interferences of components of the sample with the antibodies [2].

Cross reactivity

Cross reactivities are the result of non-target binding and appears similar to non-specific binding. Unlike non-specific binding, however, when one talks about cross reactivity the cross reacting substance is known and its cross reacting properties can be proven e.g. by measurement of the competing concentration of the cross reacting species [5]. Cross reactivity is the ability of the antibody to bind structures other than the target analyte (fig. 3I, 3J and 3K). Often, these structures are similar to the analyte such as metabolites or chemical substances with a similar molecular structure. Proteins with evolutional homology of amino acid sequence or similarity in tertiary structure can cross react too. Cross reactivities play a key role in many competitive assays, because only one antibody is used [1, 4]. For these assays, it is part of the validation to identify and to quantify possible cross reactors experimentally [5].

Cross reactivities can also play a major role in the detection of proteins in Western blots or in immunohistochemical applications. Cross reactivity can result in staining of additional bands in Westerns or cell structures, without knowing the exact molecular reasons for this unwanted binding. In Western blots, sometimes the additional bands simply represent protein fragments which originate from the "normal" degradation process. But in some cases it is important to look closer at cross reactivities caused by the primary or secondary antibody. For any scientific publication it is necessary to verify the reasons for unexpected bands and signals anyway.

Matrix effects

The least defined term is the "matrix effect". Matrix effects are the sum of all negative effects of all components within a sample, which can affect the determination of the target analyte [6]. If the exact molecular cause of such an effect is unknown, but can be related to the composition of the sample to be determined, one speaks about a matrix effect. There is a smooth transition to all other negative effects. Matrix effects can be caused by Anti-Animal-Antibodies, heterophilic antibodies, endogenous interfering substances or influences like viscosity, pH or salt concentration.

There are negative effects that are restricted to medical and diagnostics assays. These effects are based on interfering substances present in human specimen like plasma, serum or tissue samples. Since the results of these assays are the basis of patient therapies, interferences and false results are severe.

Anti-Animal-Antibodies

Human Anti-Animal-Antibodies (HAAA) are of the IgG, IgA, IgM or IgE type and are formed as an immune response after contact with animal immunglobulins. HAAAs are well known from diagnostic assays. Studies report that up to 80% of all samples contain HAAAs. The concentrations can be very high, reaching levels of several milligrams per milliliter [7].

Human-Anti-Mouse Antibodies (HAMA) are the best known interfering antibodies in immunoassays. HAMAs are human antibodies which are relatively specific and which can bind mouse antibodies with a middle affinity up to, in some rare cases, a high affinity. One of the reasons for the development of these antibodies can be non-human therapeutic antibodies which are administered as drugs (e.g. in cancer therapies). After therapeutic medication the human immune system reacts to these foreign antibodies and begins to produce antibodies against these mouse antibodies. Therefore, HAMAs interfere with immunological methods which include mouse antibodies. In sandwich ELISAs based on monoclonal mouse antibodies, capture and detection antibody will be bridged (fig. 4F), resulting in a false

positive signal. Due to the sequence homology between antibodies of different species, HAMA-containing sera may disturb assays which contain antibodies from other species.

Drugs aren't the only reason for the development of HAMAs. Contact with domestic animals over several years enhance the formation of anti animal antibodies that either bind to antibodies of a single species (e.g. rabbit, mouse, dog, hamster) or binding to antibodies of several species with different affinities. Some interfering antibodies can bind to the Fc portion of the antibody, while others bind to the Fab-fragment resulting in reduced binding of the analyte or even completely preventing the formation of any real complexes. The consequence is a false negative measurement (fig. 4G and 4H). The ability of HAAAs to bind the Fc-fragment is called anti-isotypical interference. In contrast, anti-idiotypical interfering antibodies bind the highly variable, Fab portion of the antibody [7].

Heterophilic antibodies

According to Taber's Medical Dictionary, 'heterophilic antibodies are antibodies which bind other antigens than the specific antigen'. Heterophilic antibodies can be of the IgG, IgM, IgA or IgE type. The IgM type plays a key role in sera from rheumatic patients. These sera do contain so called rheumatic factors in high concentration. Rheumatic factors are IgM type antibodies which bind to Fc-fragments of human antibodies, and therefore they may bind to the Fc-fragments of antibodies of other species in the assays as well. Rheumatic sera lead to a linkage of capture and detection antibodies with the consequence of false-positive signals. This reflects the general interfering mechanism of heterophilic antibodies. The effect of the rheumatic sera is similar to the effect of HAAAs. The difference between HAAAs and heterophilic antibodies is their formation. The latter aren't formed upon contact with animal immunglobulins, but rather they are multispecific antibodies of the early immune response or interfering antibodies with unknown immunological origin [7].

Interference by HAAAs or by heterophilic antibodies have been known now for more than 30 years. In general, the interfering antibodies are weakly binding antibodies [7], which predominantly disturb assays that, due to the low concentration of the analytes, require a low dilution of serum or plasma specimens [8]. Addition of blocking substances to the sample buffer, e.g. non-specific sera, antibody fragments or high concentrations of animal immunoglobulins, are able to reduce the negative effects of the HAAAs or heterophilic antibodies by competition, but don't always prevent them [7].

Interference caused by endogenous components of the specimen

Even naturally occurring proteins found in specimens can interfere with immunoassays. Some well known interfering substances in human sera are albumins, complement factors, lysozymes and fibrinogen [4]. Since analytes of low molecular weight can bind readily to albumin, this reduces the accessibility of the antibody to the analyte. Numerous hormones are bound to transport proteins, which may lead to difficulties as well. The binding ability of certain proteins is a substantial part of their biological function, e.g. albumin, complement and C-reactive protein (CRP). Because these proteins are natural receptors for many substances, non-specific binding or even cross reactivity is possible which complicates the recognition of certain analytes in an assay similar to antibodies. Endogenous proteins can bind as interfering factors to antibodies (fig. 2C, 3I-K) or mask the target analyte (fig. 5L). For example, lysozyme binds non-specifically to any proteins with a low isoelectric point. Therefore, antibodies which have

an isoelectric point of approximately 5, can be bound and form a bridge between capture and detection antibody [4].

One other important aspect, which should be mentioned, is the interference by strongly fatty specimens, because some analytes are fat-soluble and the binding between antibody and analyte can be affected by lipids.

Avoiding the interference by applying novel immunoassay buffers – examples from the practice

In most cases, the problems in many immunoassays are caused by low to medium affinity bindings. The best known strategy to circumvent the negative effects is an optimised blocking procedure. To get the systems running many blocking solution were developed. Most of them can be called 'very creative', but rather lack real good results in practice. The larger the analyte, the easier is the blocking. Small analytes often require a more efficient blocking. The optimal blocking buffer shall be a generally applicable solution for most immunoassays and shall give reliable results. Only such a multi-purpose solution can help saving time and money for optimizing and developing immunoassays. This holds true especially if expensive antibodies or difficult-to-prepare samples are applied. Casein-based blocking solutions have proven to be very efficient. But preparing such a solution with consistent blocking efficiency requires a great deal of time and experience. The reason is simple. Simply solubilizing casein doesn't give a good blocking solution. That kind of casein blocker is available from many suppliers, but the results in assays are not of the same quality like they should be. Literature describes and practice shows that casein works best, if it is cut into fragments of different molecular weights. Nowadays, a chemical modification during the manufacturing process allows the production of casein solutions with reproducible and reliable results.

Replacement of an unsuitable blocking reagent in immunohistochemistry makes the interpretation of an experiment with osteoblast culture possible (fig. 6). On the first day, a freshly prepared osteoblast culture shows no or a very weak expression of the extracellular matrix protein osteocalcin. By applying a novel casein-based blocking reagent (Blocking Buffer I, AppliChem) in combination with anti-osteocalcin (monoclonal, TaKaRa), the actual expression is correctly detected (fig. 6 upper left). Standard blocking with BSA leads to a completely false-positive result (fig. 6 upper right). With time, the cultured osteoblasts build up the extracellular matrix and osteocalcin is synthesized. The expected increase in osteocalcin expression can be correctly monitored by using Blocking Buffer I. The staining of the cultures intensifyes with time (fig. 6 lower left).

In many cases the substitution or optimisation of the blocking reagent alone is not sufficient, because the blocking agent has limited influence on all the different negative effects. The solution to combating the one common feature of many interfering substances, i.e. low to medium affinity binding, was used to develop a new buffer.

CrossDown Buffer (AppliChem) capitalizes on the fact that the binding of interfering substances is weaker than the specific binding of the target analytes. It eliminates low and medium affinity binding, without negatively affecting high affinity binding and high specificity. Figures 7 to 10 show different examples of typical interference effects in immunoassays that are prevented by the use of CrossDown Buffer.

Figure 7 shows a Western blot with high background. One of the typical daily problems in many labs. Only the substitution of the blocking reagent and additionally substitution of the antibody dilution buffer led to an analyzable result: Myostatin (GDF-8; 12 kDa) from mouse myoblasts (C2C12) was blotted on nitrocellulose NC45 (Serva) and detected with anti-GDF-8 (Santa Cruz). Originally, blocking was performed with 2% non-fat dried milk powder and 1% BSA in TBS. As antibody dilution buffer 0.3% BSA in TBS was applied and detection was done with ECL (Amersham). Applying the conventional protocol, the bands are hardly visible. With the substitution of Blocking Buffer I and CrossDown Buffer as new antibody dilution buffer, a significant reduction of background is achieved (fig. 7).

Positive results in a protein chip application are shown in fig. 8. CrossDown Buffer reduced a high background and improved the signal-to-noise ratio from 3.4 to 17.3. In this experiment different polyclonal anti EPIL antibodies (EPIL – early placenta insulin like growth factors) were tested for their suitability. The purified antibodies were immobilized on aminosilane-functionalized micro-array slides using a spotter (GMS 417) at a concentration of 500 µg/ml in a volume of 1.8 nl/spot. Afterwards, 2 ml supernatant of an EPIL-over-expressing cell line (SKBR3) were mixed with the dye Oyster650P (Denovo Biolabels) and all proteins of the mixture were labeled. The incubation on the slide was carried out at a dilution of the medium : buffer 1 : 20 with CrossDown Buffer in comparison to PBS. After washing of the slides they were analyzed with a fluorescence scanner (GMS 418) and the data were evaluated with ImaGene (Biodiscovery Inc.). The use of CrossDown Buffer resulted in a clear reduction of the background signal, allowing the selection of antibodies in terms of their suitability to detect EPIL.

An example of the impact of a matrix effect on an ELISA is shown in figure 9. With this model assay a

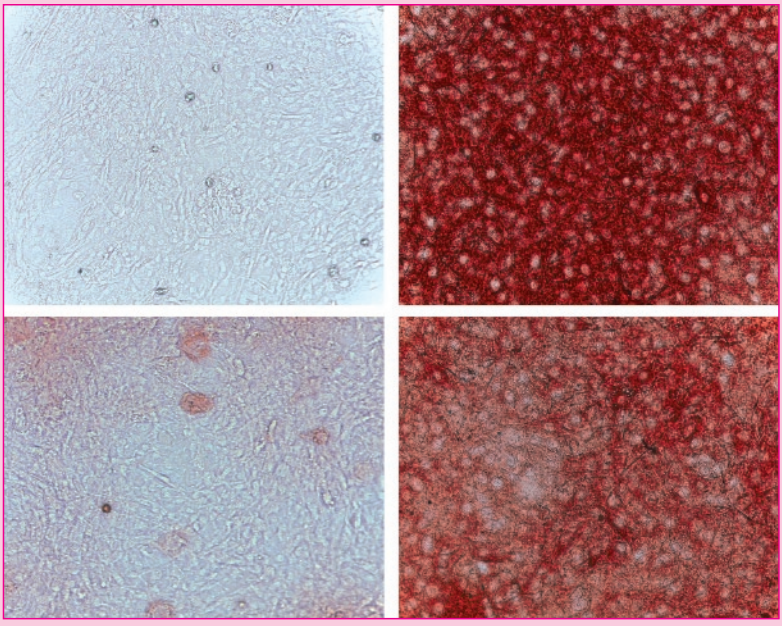

Fig. 6 Osteoblast culture for detection of osteocalcin. Whereas blocking with BSA gives a wrong result (upper right), switching to a novel casein-based blocker (Blocking Buffer I, AppliChem) shows correct results (upper left). The time course of expression can be shown correctly (lower panels).
(images by PD Dr. Wiesmann, University of Münster, Germany)

Fig. 7 Western blot. Left side without and right side with Blocking Buffer I and CrossDown Buffer. Detection of myostatin in mouse myoblasts with anti-GDF-8 as primary and rabbit anti-goat IgG-HRP as secondary antibody on a nitrocellulose membrane NC45. (Dipl. Biol. S. Siewert, University of Ulm, Germany)

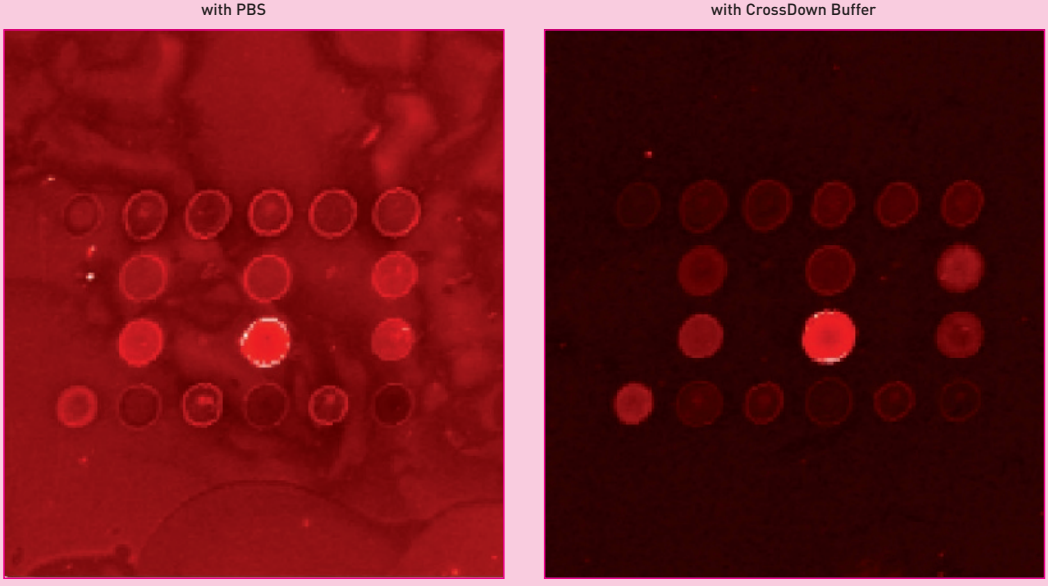

Fig. 8 Reduction of a non-specific interaction of the detection antibody with the array surface by the use of CrossDown Buffer. Signal-to-noise could be increased from 3.4 to 17.3. (Dipl. Chem. N. Dankbar, University of Münster, Germany)

matrix effect was induced systematically. The assay was performed by Candor Bioscience GmbH, a company, which develops and validates assays for pharmaceutical research and diagnostic applications. Rabbit serum was used as a matrix and spiked in defined concentrations with human C-reactive-protein (CRP, Biotrend). As capture antibody, Clone C2 was used (Biotrend, 1 µg/ml coating concentration in PBS) and for detection, the biotinylated antibody from Clone C6 (Biotrend, working concentration 2 µg/ml) was applied. The spiked serum samples were diluted either with a PBS-BSA buffer or with CrossDown Buffer 1 : 2 and measured by ELISA. Detection was carried out with NeutrAvidin™ - conjugated horseradish peroxidase (Pierce, working concentration 0.05 µg/ml in PBS-BSA buffer) with ImmunoPure®TMB-substrate (Pierce).

A matrix effect, whose exact molecular reason is not known, leads to a calibration curve with low sensitivity. Due to its physiological function, CRP is able to bind many proteins and substances (scavenger function of CRP), probably causing a significant reduction in the accessability of the epitope by the antibody. Presumably, an interfering effect as shown in figure 5L takes place, although interfering effects as shown in figure 3I-K can not be excluded. Again, CrossDown Buffer prevented the binding of CRP to endogenous substances of the rabbit serum and thus improved the sensitivity of the calibration curve by the factor of 3 (fig. 9).

The ELISA shown in fig. 10 is an example, where the substitution of sample and antibody dilution buffer by CrossDown Buffer was sufficient to achieve a good result. As antigen a lysate of human kidney carcinoma cells were immobilized and a serial dilution of two immun sera in repeat determination (1:50 to 1:36450) A–G loaded in columns 1–4. The corresponding preimmun sera (1 : 50) were pipetted in lane H. Blank values are in column 5. The result of using the standard buffer (PBS/NaCl/Tween® 20) in contrast to the new CrossDown Buffer is self-evident. It leads to a better sensitivity by reducing the level of detection (LOD) from 0.051 to 0.022 and the level of quantification from 0.152 to 0.065 and enlarging the measurement range. The improvement can be explained by the elimination of the false-positive signals of the preimmun sera and the reduction of the background.

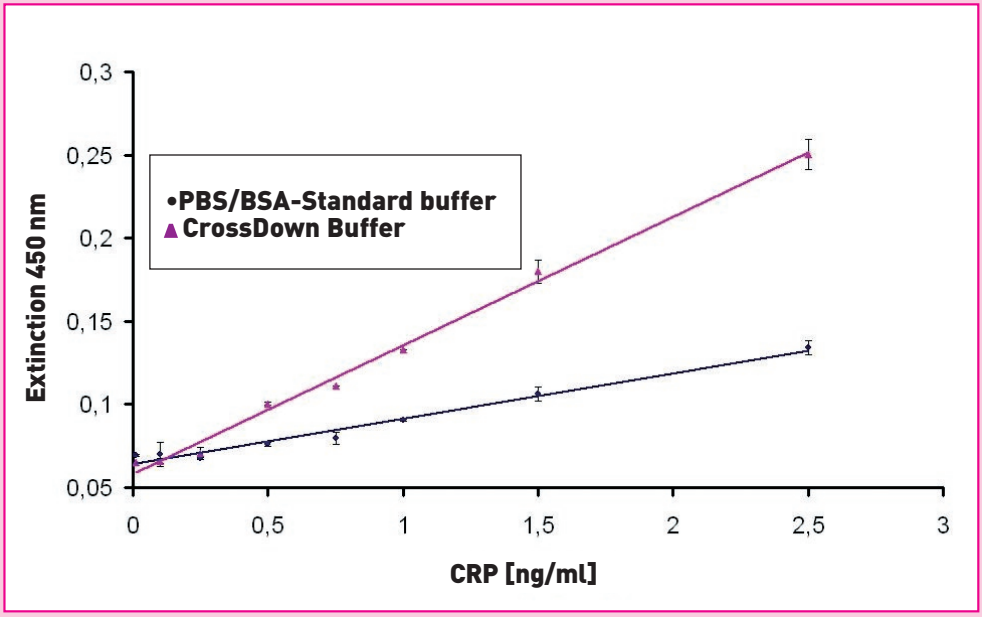

Fig. 9 ELISA of CRP in rabbit serum (developed by P. Rauch, Candor Bioscience GmbH). CrossDown Buffer improved sensitivity by avoiding a matrix effect.

Conclusion

The phenomenon of interference in immunoassays is as old as the application of antibodies for bioanalytical and diagnostic purposes. During the last 30 years numerous molecular causes were found and the mechanism of interference investigated which led to the development of prevention strategies. At today's state of the technology, many interference effects can be minimized and innovative buffers for immunoassays make an essential contribution to it. In fact one can say, that "good solutions" for these problems have been developed. It is new that the same sample and antibody dilution buffer allows to minimize different interference effects with different molecular principles at the same time. CrossDown Buffer is applicable for different immunoassays.

The results shown here cover just a part of the different negative effects in different methods, which could be minimized or even avoided with this novel buffer. In addition, non-specific binding in immunohistochemical applications and false-positive binding in immuno-PCR can be prevented. The novel Blocking Solution I, which is manufactured in reproducible quality with its wide spectrum of fragments of different molecular weights, can help to increase the efficiency of these methods. Taken together, costs and time for optimizing assays can be avoided and reduced, respectively, as well as reliability improved.

Literatur

[1] Miller, J.J. (2004) Clinical Laboratory International **28**, *(2), 14-17*

[2] Kusnezow, W., Hoheisel, J.D. (2003) J. Mol. Recognit. **16**, *165-176*

[3] Patton, W.F. (2000) Electrophoresis **21**, *1123-1144*

[4] MacBeath, G. (2002) Nat. Genet. **32**, *526-532*

[5] Miller, J.J., Valdes, R.Jr. (1992) J Clin Immunoassays **15**, *97-107*

[6] Wood, W.G. (1991) Scand. J. Clin. Lab. Invest. Suppl. **205**, *105-112*

[7] Kricka, L.J. (1999) Clinical Chemistry **45**,*(7), 942-956*

[8] Span, P.N., Grebenchtchikov N., Geurts-Moespot, J., Sweep, C.G.J. (2003) Clinical Chemistry **49**,*(10), 1708-1709*

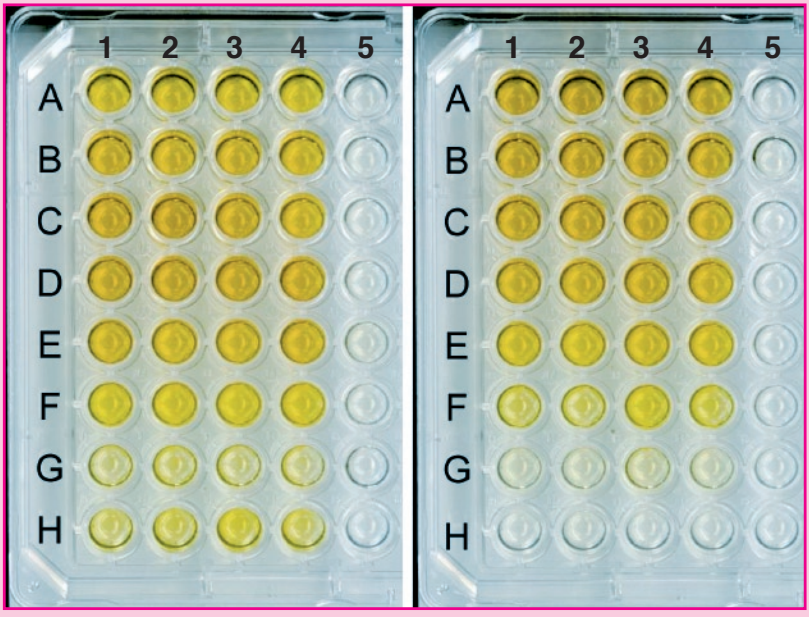

Abb. 10 ELISA with lysates of a human kidney-cell carcinoma. Left side PBS/NaCl/Tween® 20 and right side with novel CrossDown Buffer (AppliChem) Lane **A-G** serial dilution of immun sera; Lane H: preimmun sera; Column 5: blank. (Dr. Specht, Para Bioscience GmbH).

Without Blocking ... No Result!

This is a short message describing the necessity to block surfaces in immunoassays.

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Dr. Tronhung Quang, Dr. Rainer Klocke, Prof. Dr. Sigrid Nikol university hospital Münster,
Dr. Christoph Specht PARA Bioscience GmbH

Many different blocking protocols exist and they work in various assays - sometimes better, sometimes worse. However, universal blocking solutions do not exist.

All kinds of immunoassays require blocking to prevent non-specific binding of antibodies or components of the sample to surfaces e.g. ELISA plates or Western blot membranes. Otherwise, this binding would lead to a strong background, falsify or even destroy results. Efficient blocking means no areas on surfaces are available for non-specific binding. That's the theory. In practice, the devil is in the details. A review of the literature shows that for every detection method using antibodies, several hundred blocking protocols exist describing variations of blocking solutions based on different blocking reagents. They all have in common that certain molecules are present in vast excess to cover the entire surface.

Frequently used blocking reagents contain BSA, gelatin from fish, non-fat dried milk, casein or synthetic molecules. Unfortunately, the optimum reagent has to be determined for each new assay, since they all have certain restrictions when used with real samples such as blood, serum, cell lysates or tissue sections.

Ideal Blocking

What properties are a must for the ideal blocking reagent? First, it has to completely cover the surface and this is achieved best, if it contains molecules of different sizes where large and small gaps will be covered simultaneously. Secondly, the blocking reagent shall not react with or bind to any components of the sample or the antibodies. BSA blocking reagents pose special problems since many analytes are coupled to BSA to achieve a better immunization. It is no wonder that some antibodies will bind with high affinity to BSA. In this case, blocking would lead to an even increased background due to the reaction of antibodies with the blocking reagent.

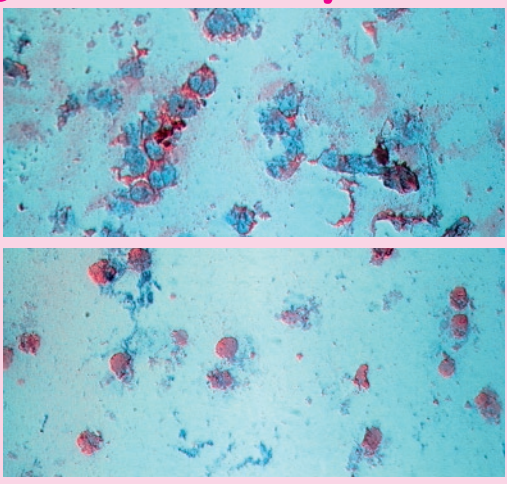
Highly purified Casein-based reagents have proven to meet most of the important criteria of the ideal blocker but the preparation of such a reagent requires experience of a skilled person. Hydrolysis of casein must be performed over many hours and any mishandling may lead to precipitation of casein.

Blocking Buffer I - *ready-to-use*

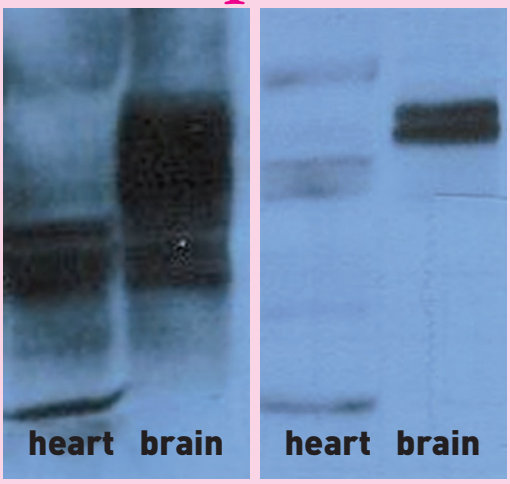
AppliChem now offers Blocking Buffer I which meets all criteria. It is based on highly purified, chemically modified casein with an optimum distribution in terms of fragment size. This ***ready-to-use*** buffered solution requires no assay optimization and is stabilized with ProClin® 300 instead of toxic additives like thimerosal or sodium azide.

Nevertheless, even effective blocking may not prevent some background, caused by matrix effects or cross reactivity. Additionally applying AppliChem's CrossDown™ Buffer shall reduce or even abolish negative effects in different immunoassays such as immunohistochemical staining, Western blots or ELISAs.

Just theory? This is the practice!



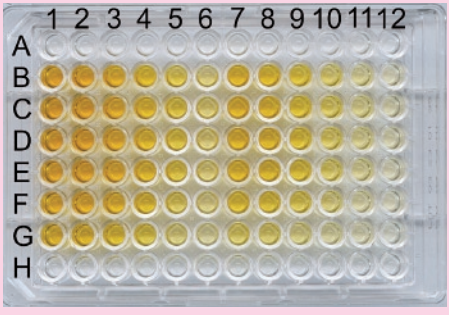
Immunohistochemistry
The antigen Nestin was detected by ABC-immunocytochemical staining with alkaline phosphatase on Cytospin preps of the neuroblastoma cell line SK-N-LO. While a standard blocking buffer based on 1 % BSA in PBS stained large parts of the whole surface (upper panel), using the *ready-to-use* Blocking Buffer I led to a reduction of background staining (lower panel). Correct staining of the cytoplasmic Nestin is now clearly separated from the hematoxylin staining of the nucleus.



Western-Blot
Detection of the antigen CaMK II in lysates of heart and brain tissue of C57Bl/6 mice by Western blotting. After separation by SDS-PAGE, proteins were transferred to a nitrocellulose Optitran BA-S 83 membrane (Schleicher & Schuell) and detected by ECL. The primary antibody was a monoclonal anti-CaMK II antibody (BD Bioscience), and the secondary antibody a polyclonal HRP-conjugated antibody (Santa Cruz). Blocking with 5 % non-fat dried milk in TBS-Tween® is shown on the left side. This Western blot cannot be interpreted. A combination of Blocking Buffer I and CrossDown™ Buffer as antibody dilution buffer for the primary and secondray antibody (right side), made the identification of the bands possible.

with PBS

with CrossDown



ELISA
This ELISA (developed by PARA Bioscience, Gronau, Germany) detects immunoglobulins from guinea pig and is used for immunotoxicological studies with guinea pigs. The false-positive binding observed in the control lane **A1–A12** and the blind values (lane **H1–H12**) prevents interpretation of the assay.

Use of CrossDown™ Buffer abolishes false-positive binding and allows a concentration-dependent detection (lanes **B to G1–6** and **B to G7–12**). The capture antibody Goat-anti-guinea pig-IgG **F(ab')2** and detection antibody Goat-anti-guinea pig-IgG F(cγ) biotinylated were from Jackson ImmunoResearch Laboratories, Inc. (concentration range each 0.31–10 µg/ml in PBS). Guinea pig IgG was diluted either in CrossDown™ Buffer or PBS (columns 1–6 50 ng/ml; columns 7–12 10 ng/ml). PBS-BSA buffer was used as a blocking buffer and detection was carried out with streptavidin-peroxidase (Sigma) and ortho-phenylenediamin (Sigma).

tips & tricks

Which substances may be added to antibody-containing solutions as preservatives?

Frequently, in biomedical laboratories sodium azide (final concentration 0.02–0.2 %) or Thimerosal (final concentration 0.005 %) are added to reagents. Both substances are toxic and less harmful alternatives are wanted. For such applications, ProClin® 300 may serve as a substitute, if growth of bacteria and fungi/molds has to be prevented.

Does it make sense to add albumin?

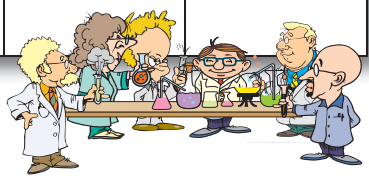
Principally, it is good to add albumin, because albumin stabilizes antibodies. For long-term storage of antibodies in solution, we recommend the use of our Antibody Stabilizer (prod. no. A7148 and A7135), because they contain special ingredients stabilizing the structure.

Is it possible to store antibodies in 50 % glycerol?

The answer is Yes, if you want to freeze antibodies. In most cases this procedure works better than without glycerol. Glycerol acts like antifreeze preventing the formation of ice crystals, keeping the product liquefied?

If you want to freeze antibodies, we recommend testing the performance of the antibody after thawing, because some antibodies cannot be frozen without losing their activity. In case the test is positive, prepare small aliquots and freeze. This is the best way to store antibodies for long term storage.

Selection Guide	ELISA, EIA, RIA	Western Blot	Protein Array	Immunohisto-chemistry	Immuno-PCR
CrossDown Buffer	■	■	■	■	■
Sample Buffer T+	■	■	■	■	■
Sample Buffer T-				■	
Blocking Buffer I	■	■	■	■	■
Blocking Buffer II EGrade	■	■	■		■
Blocking Buffer III BSA	■	■	■		■
Washing Buffer TrisT+	■	■	■	■	■
Washing Buffer TrisT-				■	
Coating Buffer	■		■		■
Stripping Buffer I		■			



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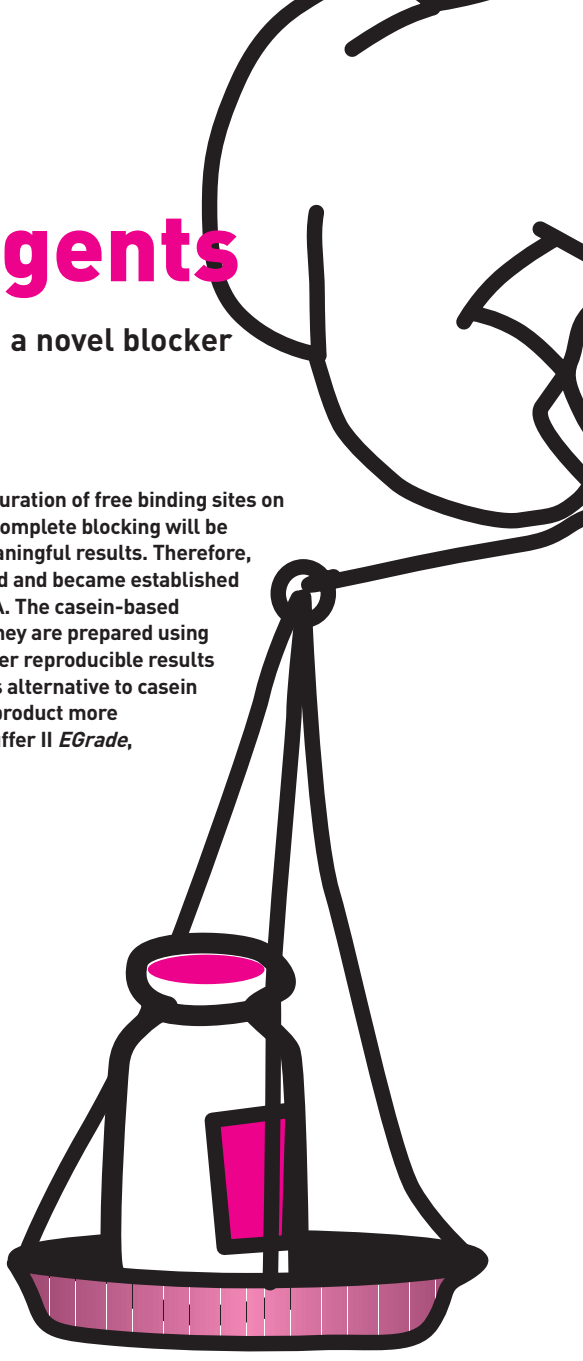
Comparing Blocking Reagents

Quality and economy now combined in a novel blocker

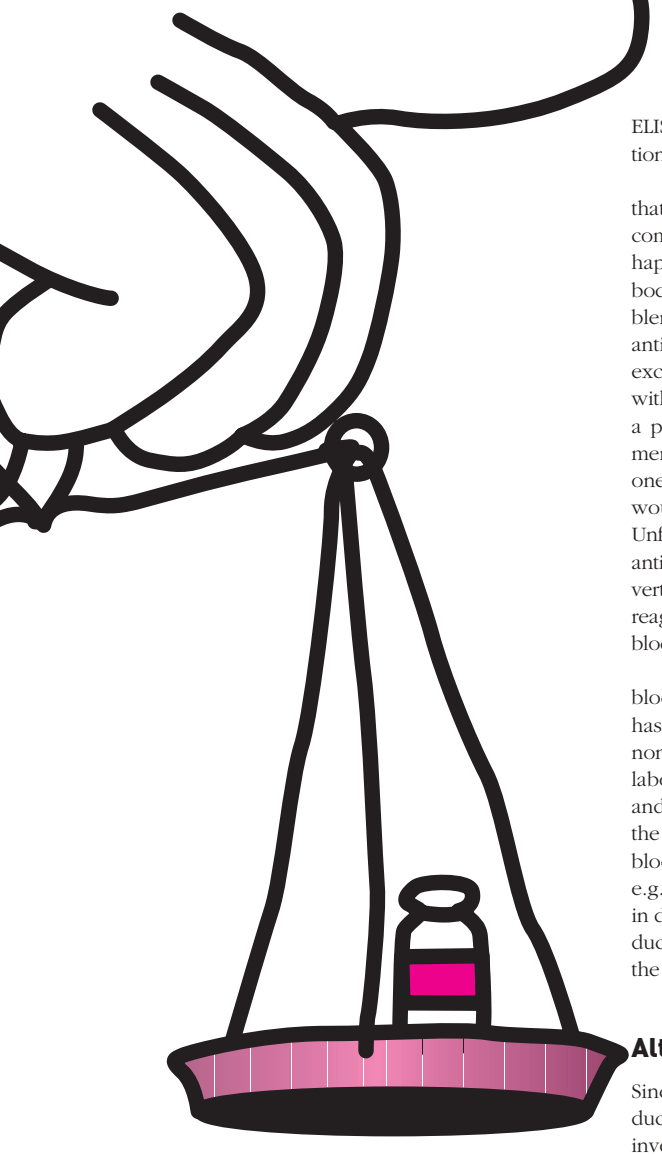
Dr. Wolfram H. Marx, AppliChem



Blocking in immunoassays is an important step, i.e. the saturation of free binding sites on surfaces of ELISA wells or Western Blot membranes. Noncomplete blocking will be cursed with high background and either useless or nonmeaningful results. Therefore, several very different methods for blocking were developed and became established especially protein-based blockers containing casein or BSA. The casein-based blockers show an unsurpassing efficiency, but only when they are prepared using casein which has undergone severalfold cleavage. Whenever reproducible results with a low standard deviation are of importance, no serious alternative to casein exists. Unfortunately, preparation is complex making this product more expensive. Now, we present a new alternative, Blocking Buffer II *EGrade*, combining quality and economy.

State of the art

If samples are rare, expensive, or the results have to be reliable, then there exists no alternative to the highly purified casein-based blocking reagents (e.g. Applichem's Blocking Buffer I). The only option available is based on ultrapure BSA and in many test systems, efficiency is comparable. Differences appear in terms of standard deviations when real samples are measured (determined as coefficient of variation (CV) for multiple measurements). These differences are mainly due to differences in molecular size of the blocking agent. The relatively large BSA leaves gaps on the support surface which will be covered by smaller molecules coming from the sample. Most of these substances do not influence the results. Some do interact with e.g. detection antibodies or the label such as peroxidase, phosphatase or fluorescent dyes. As soon as interactions occur, they lead to erroneous results. The difficulty is that one cannot predict when it will happen, how strong the interference will be and whether deviations will shift results up or down. You can identify such interferences simply by observing high standard deviations, i.e. high CVs. Looking at the CV as shown in fig. 1, the problem is obvious. Figure 1 shows a sandwich ELISA, which seemed to work well by including a BSA-based blocking reagent (Blocking Buffer III BSA). But CV's tell something different. Upon first glance, the assay looks good but the results are unreliable. Not until running the same assay using AppliChem's Blocking Buffer I, is a reliable result achieved. At first view, what appears as an unimposing effect, ultimately influences the significance of all subsequent tests. In most cases, ELISAs, Western Blots or other immunoassays simply serve as tools to verify or falsify a hypothesis of the whole research project. Those who use unreliable tools, as shown for the BSA-blocked



ELISA, must be prepared that their results will be questionable and, in the present case, doubts are justified.

There is another problem arising from time to time that must not be overlooked. Over the years it has been common practice to couple small antigenes, so-called haptens, to BSA as carrier molecules to generate antibodies. It works well, is reliable and simple. The problem is that a large number of commercially available antibodies as well as antibodies generated by and exchanged between scientists not only bind the hapten with significant affinity but also BSA. This wouldn't be a problem if the producers of such antibodies would mention the method of immunization. In those cases, one would be aware of the potential interference and would not choose a BSA-based blocking reagent. Unfortunately, the exact opposite is reality and research antibodies do not include this essential information. Nevertheless, in most cases BSA is a very good blocking reagent. It is cheaper than optimized casein-based blocker, even if the "BSA" purification is laborious.

The big disadvantages of good protein-based blockers is the high costs and the search for alternatives has resulted in several varieties. Gelatin from fish or non-fat dried milk are just two to mention. In some laboratories and assays they meet the requirements and, in many other assays they constantly fail. Due to the uncontrollable quality of these raw materials, these blocking reagents are not used in medical research, e.g. in pharma assays, preclinical or clinical studies or in diagnostics, since reproducibility is not given. Reproducibility of an assay depends on constant quality of the raw materials.

Alternatives wanted

Since the desired alternative needs to be of high, reproducible quality but at low cost, synthetic blockers were investigated and Tween® represents the first, best known and simplest solution. Other synthetic blockers are also availavle at low prices as well. While consistency of raw materials may be optimal, one problem couldn't be solved: reproducibility of results thereby limiting their use. Considering that the total costs of blocking are just a small part of the total costs of an assay, it is obvious why very few scientists would trade quality for marginal savings.

Now, AppliChem presents THE new alternative for efficient and cost-effective blocking in immunoassays. Blocking Buffer II *EGrade* is manufactured to DIN ISO 9001:2000 quality requirements, free of any serum proteins, and peptide-based. Since the synthesis of the peptides is controlled, blocking is uniform and reproducible from batch to batch. The costs are comparably lower than for protein-based blockers, while quality is acceptable – in contrast to synthetic blocker.

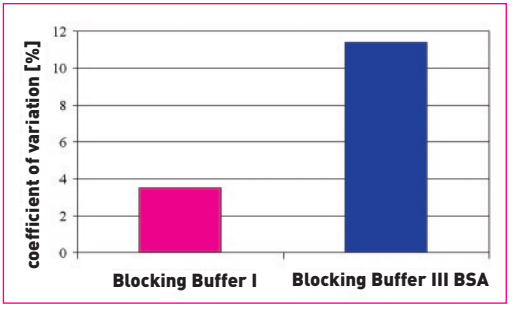


Fig. 1 Comparison of the coefficient of variation (CV) of an ELISA with blood samples. The ELISA was performed once with Blocking Buffer I and once with Blocking Buffer III BSA under identical conditions. To determine the CV a complete ELISA plate was measured (n=96).

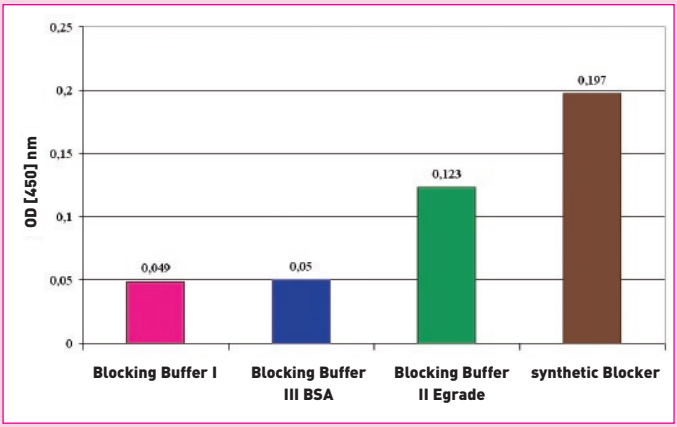


Fig. 2a OD-values for blocking over night with different blocking reagents.
This is a modified analyte-free assay originally developed by Steinitz & Baraz (2000), measuring the influence of blocking without negative effects of real samples.

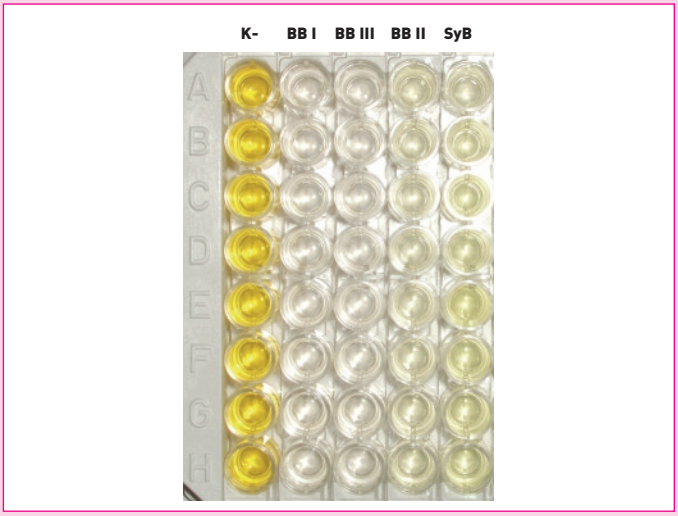


Fig. 2b Image of the ELISA plate described in **fig. 2a**. The left column is the control without blocking (K-). The order of blocking reagents is as in **fig. 2a** Blocking Buffer I (BB I), Blocking Buffer III BSA (BB III), Blocking Buffer II *EGrade* (BB II) and synthetic blocker (SyB).

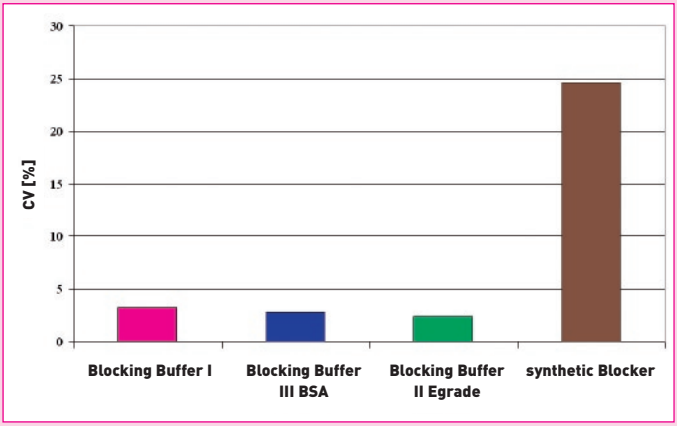


Fig. 3 Graph of the coefficient of variation (CV) of the assay from **fig. 2**.
The lower the CV, the more reliable the assay. In industrial practice, CVs of < 15 % for ELISAs with real samples are needed (e.g. according to FDA guidelines). Please note that the CVs shown here only represent effects of blocking reagents, since the assay don't includes analytes and other negative effects.

Analyte-independent comparison

Is it possible to determine the real blocking efficiency and quality of a new blocking reagent? According to the methods of Steinitz & Baraz (2000) and Steinitz (2000), a test system was established with the following changes: peroxidase was used instead of phosphatase as reporter enzyme, eight determinations instead of double values, and additional determination of the coefficient of variation (CV).

The test included Blocking Buffer I, highly purified and multiply digested casein, Blocking Buffer III BSA based on BSA, the novel Blocking Buffer II ***EGrade***, as well as a commercial, synthetic blocking reagent.

The surface of the ELISA wells were blocked by the different blocking reagents and incubated with a detection antibody (analog to Steinitz (2000), but using peroxidase) without any additional additives. After washing, the staining reaction was carried out. The lowerthe optical density (OD), the better the blocking efficiency.

Figure 2 shows blocking overnight. All variants do block. The blocking efficiency of the protein-based blocking reagent is superior, good for Blocking Buffer II ***EGrade*** and also good for the synthetic product. The graph in fig. 2a shows the ODs, fig. 2b the corresponding image of the blocked ELISA plate. The left column served as an unblocked control.

The coefficient of variation of the same experiment is presented in fig. 3. Here, the wheat separates from the chaff. At first view, the synthetic solution looks promising. Even though a simple, artificial test system without any interfering substances was used, the CV value of 25% is extremely high. Such a CV is transferred to the total CV of an assay according to the error propagation of each separate error of the assay. It is questionable whether a reproducible assay may be established with such a synthetic blocking reagent. The advantage of the novel Blocking Buffer II ***EGrade*** is clear. Blocking is highly reproducible with a constant CV, identical to the protein-based reagents.

Combining the results shown in fig. 2 and 3 allows one to draw the conclusion that in those assays, where Blocking Buffer II ***EGrade*** gives a low background, reproducabillity is seen also. This is an advantage over other alternatives like the fish gelatin, milk powder or synthetic blocking reagents. Blocking Buffer II ***EGrade*** combines economy and reproducibility for various assays, and may be regarded as a suitable alternative to the protein-based blocking reagents. The comparison according to Steinitz also proves the unique position of protein-based blockers for sensitive applications like pharma assays, clinical studies or other assays including valuable samples.

Conclusion

For many assays in LifeSciences, AppliChem offers an alternative to high cost protein-based blocking reagents. Blocking Buffer II ***EGrade*** has the potential to combine economy and quality.

Literatur

[1] Steinitz, M. & Baraz, L. (2000) J. Immunol. Methods **238**, *143-150. A rapid method for estimating the binding of ligands to ELISA microwells.*

[2] Steinitz, M. (2000) Anal. Biochem. **282**, *232-238. Quantitation of the Blocking Effect of Tween® 20 and Bovine Serum Albumin in ELISA Mircrowells.*

Sometimes, less is more!

For The Sake Of The Environment

Many reagents prepared in biomedical research labs are ideal nutrient broths for unwanted germs (bacteria, fungi). To prevent their growth, reagents are either autoclaved, sterile filtered, or antibiotics / antimycotics or toxic substances are added. One of these additives is Thimerosal, a mercury-containing molecule which is dangerous for the environment. Our original immunoasssay buffer contained this chemical too, but now we have replaced it by the nontoxic ProClin® 300. For the sake of the environment.

For Your Safety

We would like to keep you healthy so that you stay our customer. FYI: Thimerosal is classified as toxic. The lethal dose (rat, s.c.) is 9 mg/kg, compared to ethidium bromide with a lethal dose (mouse, s.c.) of 110 mg/kg. In some countries, Thimerosal is a forbidden additive.

For Better Results

Changing the composition has no negative influence on the performance of the products. With CrossDown and all other immunoassay buffers you are even in a better mood and your immunoassays show a better quality.

AppliChem

Antibody Stabilizer-PBS A7148

Stabilization buffer for long-term storage of antibodies and proteins

A7148,0050	50 ml
A7148,0125	125 ml

pH value	pH 7.4 ± 0.2
Preservative	contains 0.1 % ProClin®300
Storage	–20 °C or at 2–8°C
Stability	at –20 °C: 2 years ; at 2–8 °C: 1 year

Instructions for use

Antibody Stabilizer-PBS is made for long-term storage of proteins and antibodies at 2–8 °C in solution.
Components of *Antibody Stabilizer* conserve the structure of proteins and antibodies. Thus proteins and antibodies are prevented from losing functionality during storage.

Immediately before use *Antibody Stabilizer-PBS* should be mixed thoroughly. Just dissolve proteins or antibodies with *Antibody Stabilizer-PBS* for storage in the refrigerator.

You can also use *Antibody Stabilizer-PBS* for storage of coated ELISA plates. After blocking add *Antibody Stabilizer-PBS* to the coated plates. In this manner treated plates can be stored for a longer time in the refrigerator.

Stability of proteins and antibodies differs significantly from case to case. Stability depends on characteristics and concentration of the used proteins and antibodies. The user has to test it therefore with its own proteins or antibodies.
Antibody Stabilizer-PBS is *ready-to-use*.

Antibody Stabilizer-Tris A7135

Stabilization buffer for long-term storage of antibodies and proteins

A7135,0050	50 ml
A7135,0125	125 ml

pH value	pH 7.2 ± 0.2
Preservative	contains 0.1 % ProClin®300
Storage	–20 °C or at 2–8°C
Stability	at –20 °C: 2 years ; at 2–8 °C: 1 year

Instructions for use

Antibody Stabilizer-Tris is made for long-term storage of proteins and antibodies at 2–8°C in solution.

Components of *Antibody Stabilizer-Tris* conserve the structure of proteins and antibodies. Thus proteins and antibodies are prevented from loosing functionality during storage.

Immediately before use *Antibody Stabilizer-Tris* should be mixed thoroughly. Just dissolve proteins or antibodies with *Antibody Stabilizer-Tris* for storage in the refrigerator.

You can also use *Antibody Stabilizer-Tris* for storage of coated ELISA plates. After blocking add *Antibody Stabilizer* to the coated plates. In this manner treated plates can be stored for a longer time in the refrigerator.

Stability of proteins and antibodies differs significantly from case to case. Stability depends on characteristics and concentration of the used proteins and antibodies. The user has to test it therefore with its own proteins or antibodies.
Antibody Stabilizer-Tris is *ready-to-use*.

Blocking Buffer I — A7099

Solution for blocking unspecific binding sites for ELISA, EIA & Western Blots

A7099,0050	50 ml
A7099,0125	125 ml
A7099,0500	500 ml

Composition	low-molecular weight highly purified casein with NaCl and Tween®
pH value	pH 7.2 ± 0.2
Preservative	contains 0.1 % ProClin®300
Storage	–20 °C or at 2–8 °C
Stability	at –20 °C: 1 year, repeated freeze / thaw cycles are possible at 2–8 °C: 6 months

Instructions for use

Blocking Buffer I saturates free binding capacities on plastic consumables and other surfaces like ELISA plates and blotting membranes. Thus a reduction of unspecific binding on surfaces can be achieved.

Efficiency of blocking is significantly improved in comparison to standard blocking procedures by a special production method, which leads to casein molecules with many different molecular sizes. *Blocking Buffer I* can be used in ELISA, EIA, RIA, Western blotting, immuno-PCR, protein arrays as well as immunohistochemistry.

Immediately before use the buffer should be mixed thoroughly.
Blocking Buffer I is *ready-to-use*. Repeated freezing and thawing is possible. After immobilisation of capture antibody or target protein *Blocking Buffer I* is applied without dilution in wells or on membranes. Incubation time has to be adopted depending on surface characteristics by the user.
We recommend blocking over night at 4 °C, but in many cases shorter incubation is also promising.

After blocking the surface has to be washed with *Washing Buffer* to make it useable for the next working steps.

	Blocking Buffer I	Other blockers
Background reduction	**Extremely efficient** background reduction – even in critical assays	Commonly known background reduction
Usability	For use in **all immunoassays**	Some products only for use in ELISA or Western blotting, many different specialised products
Ease of application	***Ready-to-use***	For some products pre-dilution with other buffers recommended
Usability with different detection methods	**Usable with all common detection methods** very good results with peroxidases, phosphatases and fluorescent labels	Usability depends on product, negative quenching with fluorescent dyes has to be checked with some products
Effects on validation (e.g. for new FDA-guidance for industry)	**Positive effects, variations decrease,** background effects are avoide, validation criteria are met easily	Normal effects on validation, no decrease in variations shown
Storage and transportation	Cooling and freezing for long-time storage, repeated freezing and thawing possible, but **no cooled transportation needed**	Cooling or freezing for long-time storage, cooled transportation for many products recommended

Blocking Buffer II *EGrade* A7516

Solution for blocking unspecific binding sites for ELISA, EIA, Western Blots, Protein Arrays, Immuno-PCR	A7516,0125	125 ml
	A7516,0500	500 ml

Composition	peptide-based blocking buffer; free of serum and BSA, phosphate-free
pH value	pH 7.0 ± 0.2
Preservative	contains 0.1% ProClin® 300
Storage	–20 °C or 2–8 °C Immediately before use the buffer should be mixed thoroughly
Stability	at –20 °C: 1 year, repeated freeze / thaw cycles are possible at 2–8 °C: 6 months

Blocking Buffer II *EGrade* is the most economic solution (Economical Grade) for blocking of unspecific binding sites. It is THE cost-effective alternative to the casein-based Blocking Solution I. • effective blocking • simple and economically • BSA-free

Instructions for use

In many assays, *Blocking* Buffer II *EGrade* prevents nonspecific and unwanted binding to surfaces. *Blocking* Buffer II *EGrade* saturates free binding sites on the surfaces of e.g. microtiter plates (plastic consumables), Western blotting membranes or slides, avoiding undesired binding of analytes or detection antibodies to surfaces. This leads to significantly reduced background and improved sensitivity of the assay. Suitable for many assays, *Blocking* Buffer II *EGrade* represents an excellent alternative to BSA-based blocking solutions. Problems with interactions and cross-reactivities arising from BSA, which are present in many blocking solutions, are avoided by the use of *Blocking* Buffer II *EGrade* , as it is free of serum proteins such as BSA.

In case satisfying results cannot be obtained with the *Blocking* Buffer II *EGrade* , e.g. if your assay measures analytes in plasma, serum or tissue specimen, we strongly recommend using our Casein-based product *Blocking Solution I* instead (A7099).

Blocking Buffer II *EGrade* is a *ready-to-use* reagent and applied like common blockers. Use *Blocking* Buffer II *EGrade* undiluted for incubation of surfaces to saturated free binding sites in non-problematic assays.

Background and nonspecific binding can not only occur at surfaces, but also between antibodies and components of complex specimen. In this cases only an assay buffer, which is used for the immunological detection reaction, can lead to satisfactory results. Therefore we recommend using *CrossDown Buffer* (order no. A6485). *CrossDown Buffer* acts as a filter for binding, which doesn't affect high affinity binding in any way, but depletes nonspecific binding and interference like matrix effects and cross reactivities.

Saturation / Blocking

microtiter plates

1. If the plate was treated with reagents containing detergents please wash the plate 3 times in a wash buffer free of detergents (e.g. Washing Buffer TrisT-, Prod. No. A7137). If you have only used *Coating Buffer* (Prod. No. A7136 or A7150) aspirate *Coating Buffer* or empty plates by wrapping firmly onto paper cloth.
2. Add 200–300 µl *Blocking* Buffer II *EGrade* to each well. Incubate at room temperature for 1–4 hours or overnight (mostly one hour is quite enough). Duration of blocking can be minimised by shaking the plate at 600–900 rpm. Duration of blocking depends on characteristics of used surface and has to be tested individually.
3. Aspirate *Blocking* Buffer II *EGrade* or empty plates by wrapping firmly onto paper cloth. Wash 3 times in wash buffer containing a non-ionic detergent, e.g Washing Buffer TrisT+ (Prod. No. A7158).

membranes

1. If the membrane was treated with reagents containing detergent please wash the membrane 3 times in a wash buffer free of detergents (e.g. Washing Buffer TrisT-, Prod. No. A7137).
2. Incubate membrane in *Blocking* Buffer II *EGrade* at room temperature for 1–4 hours or overnight (in most cases one hour is enough). The time for blocking depends on the characteristics of the membrane used and has to be tested individually.
3. a) Wash membrane 3 times in a wash buffer containing a non-ionic detergent, e.g Washing Buffer TrisT+ (Art.-Nr. 7158).
   or
   b) add antibody for detection and continue incubation and detection.

Blocking Buffer III BSA — A7252

Solution for blocking unspecific binding sites for ELISA, EIA, Immuno-PCR, immunohistochemistry & Western Blots

A7252,0125	125 ml
A7252,0500	500 ml

Composition	Standard Blocking reagent with BSA and Tween
pH value	pH 7.4 ± 0.2
Preservative	contains 0.1 % ProClin® 300
Storage	–20 °C or at 2–8 °C
Stability	at –20 °C: 1 year, repeated freeze / thaw cycles are possible at 2–8 °C: 6 months

Instructions for use

Blocking Buffer III BSA saturates free binding capacities on surfaces of plastic consumables and other surfaces like ELISA plates and blotting membranes. Thus, a reduction of unspecific binding on surfaces can be achieved. *Blocking* Buffer III BSA is the standard surface blocker for many applications. If a blocker on basis of BSA (bovine serum albumin) is efficient enough for an assay, *Blocking* Buffer III BSA is the well-priced alternative to universally applicable and more complex blockers. *Blocking* Buffer III BSA can be used for ELISA, EIA, Western blotting, Immuno-PCR as well as protein arrays and immunohistochemistry.

Immediately before use the buffer should be mixed thoroughly.
Blocking Buffer III BSA is *ready-to-use*. Repeated freezing and thawing is possible. After immobilisation of capture antibodies or target proteins *Blocking* Buffer III BSA is applied without dilution in wells or on membranes. The incubation time has to be adopted depending on surface characteristics by the user.
We recommend blocking over night at 4 °C, but in many cases shorter incubation is also promising. After blocking, the surface has to be washed with *Washing Buffer* to prepare it for the next working steps.

If you find just the same background or unspecific binding in spite of correctly used *Blocking* Buffer III BSA, we recommend the use of *Blocking Buffer I* (Prod.-No. A7099). Efficiency of blocking is significantly improved with *Blocking Buffer I* in comparison to standard blocking procedures by the special production method of this casein containing reagent, which leads to casein molecules varying in molecular sizes. This is achieved by chemical modification from highly purified casein. Therefore, you get a maximum of safety and reproducibility. Blocking Buffer I is suited for blocking of surfaces in all immunoassays, whereas *Blocking* Buffer III BSA leads to sufficient results in non-problematic assays.

Background and unspecific binding can not only occur at surfaces, but also between antibodies and components of complex specimen. In this cases only an assay buffer, which is used for the immunological detection reaction, can lead to satisfactory results. Therefore, we recommend to use *CrossDown Buffer* (Prod.-No. A6485) for optimal results in measurements of complex and important specimen. *CrossDown Buffer* has an effect like a filter for binding, which doesn't affect high affinity binding in any way, but depletes unspecific binding and interference like matrix effects and cross reactivities.

Blocking Reagent CA A3409,0010

Blocking Reagent for Hybridization Assays and Western Blots

Storage Room Temperature

The *Blocking* Reagent CA is used in hybridization and detection procedures using non-radioactive nucleic acid probes, and for Western blots.
When immunoassays and hybridization assays, such as dot blots, Western blots, Southern blots, or Northern blots are performed, there is nonspecific binding resulting in high background. In order to reduce the nonspecific binding, *Blocking* Reagent CA is used to "block" unbound sites left after immobilization of the specific protein or after the hybridization with non-radioactive probe. The *Blocking* Reagent CA improves sensitivity and reduces background.

Note Nonfat dry milk inhibits the streptavidin-biotin interaction due to its content of biotin!

Procedure

Proteins
For blotting applications such as Western blots and dot blots, add 0.2 % (w/v) *Blocking* Reagent CA into TBST or PBST, heat to 75–80 °C in a water bath or microwave oven, and stir well until dissolved. The *Blocking* Reagent CA dissolves to give a milky solution. Use for blocking and for dilutions of antibodies.

Note Do not use the *Blocking* Reagent CA in PBST for alkaline phosphatase conjugate dilutions

Nucleic Acids
For hybridization applications add 0.2 % (w/v) *Blocking* Reagent CA to Tris-Saline buffer (100 mM Tris-Cl pH 7.5, 600 mM NaCl), heat to 60 –65°C in a water bath or microwave oven, and stir well until dissolved. The *Blocking* Reagent CA dissolves to give a clear solution. Use for blocking after the wash steps, and before incu-bation in any enzyme-conjugate solution (e.g. Streptavidin-HRP, Streptavidin-AP).

Optimization of Time Required for Blocking with the *Blocking* Reagent CA

1. Cut 7 small squares of nitrocellulose or other suitable membrane.
2. Label each square with a ball point pen in 10 minute increments (60, 50, 40, 30, 20, 10), and one without blocking.
3. Place the first square (60) in a few ml of *Blocking* Reagent CA solution, and add successive squares at 10-minute intervals.
4. Wash all squares in TBST, PBST or Tris-Saline buffer (100 mM Tris-Cl pH 7.5, 600 mM NaCl).
5. Dilute the secondary antibody or streptavidin (HRP-conjugated) in *Blocking* Reagent CA solution.
6. Incubate on shaker for 1 hour.
7. Rinse in TBST, PBST or Tris-Saline buffer three times, 10 minutes each time.
8. Detect with the Chemiluminescent Detection Kit for horseradish peroxidase (Order-No. A3417,1200).
9. Evaluate background intensity in each square. Select the incubation time that gives the lowest background.

Coating Buffer C pH 9.6 A7150

Coating buffer for capture-antibodies and proteins on surfaces A7150,0125 125 ml

Composition	Carbonate-based 10X stock solution
pH value	pH 9.6 ± 0.2
Preservative	Buffer is delivered without any preservatives, because some preservatives can interfere with the process of coating. Thus coating buffer is safe and easy useable for many applications
Storage	–20 °C or at 2–8°C Use working solution immediately!
Stability	at –20 °C: min. 3 months at 2–8 °C: 1 month

Instructions for use

Coating Buffer C pH 9.6 is made for adsorptive immobilisation of proteins and antibodies on plastics surfaces (for example microtiter plates) or other protein binding surfaces. Applications are for example ELISA, EIA, RIA and protein arrays as well as immuno-PCR.

Crystals of salt can precipitate during storage at 2–8 °C or after freezing. Therefore *Coating Buffer C* must be warmed up to room temperature and should be mixed thoroughly before preparing the working solution. This leads to dissolving of salt after shaking. The stock solution is diluted 1:10 with deionized water to get the working solution. Use the working solution the same day.

The proteins or antibodies for immobilisation are diluted in this working solution and used after mixing. The typical concentration range for standard ELISA is between 0.5 µg/ml and 2 µg/ml for capture antibodies.

Depending on the surface as well as on proteins or antibodies the useful incubation times can differ. Consequently any user should optimise its own incubation procedure. For some proteins or antibodies *Coating Buffer PBS pH 7.4* is better, for others *Coating Buffer C pH 9.6* is advantages for immobilisation. The pH-value can have an influence on the steric structure of proteins or antibodies, thus having an effect on immobilisation.
For an optimised procedure for a newly developed immunoassay we strongly recommend testing of both *Coating Buffers* in comparison.

Coating Buffer PBS pH 7.4 — A7136

Coating buffer for capture-antibodies and proteins on surfaces — A7136,0125 125 ml

Composition	PBS-based 10x stock solution
pH value	pH 7.4 ± 0.2
Preservative	Buffer is delivered without any preservatives, because some preservatives can interfere with the process of coating. Thus coating buffer is safe and easy useable for many applications
Storage	–20 °C or at 2–8 °C
Stability	at –20 °C: 1 year at 2–8 °C: min. 3 months

Instructions for use

Coating Buffer PBS pH 7.4 is made for adsorptive immobilisation of proteins and antibodies on plastics surfaces (for example microtiter plates) or other protein binding surfaces. Applications are for example ELISA, EIA, RIA and protein arrays as well as immuno-PCR.

Crystals of salt can precipitate during storage at 2–8 °C or after freezing. Therefore *Coating Buffer PBS pH 7.4* must be warmed up to room temperature and should be mixed thoroughly before preparing the working solution. This leads to dissolving of salt after shaking. The stock solution is diluted 1:10 with deionized water to get the working solution. Use the working solution the same day.

The proteins or antibodies for immobilisation are diluted in this working solution and used after mixing. The typical concentration range for standard ELISA is 0.5 µg/ml to 2 µg/ml for capture antibodies.

Depending on the surface as well as on proteins or antibodies the useful incubation times can differ. Consequently any user should optimise its own incubation procedure. For some proteins or antibodies *Coating Buffer PBS pH 7.4* is better, for others *Coating Buffer C pH 9.6* may have advantages for immobilisation. The pH-value can have an influence on the steric structure of proteins or antibodies, thus having an effect on immobilisation.
For an optimised procedure for a newly developed immunoassay we strongly recommend testing of both *Coating Buffers* in comparison.

CrossDown Buffer A6485

Immunoassay buffer for minimisation of unspecific binding, cross-reactivities and matrix effects

A6485,0050	50 ml
A6485,0125	125 ml
A6485,0500	500 ml

pH-Value	pH 7.2 ± 0.2 Phosphat-free, *ready-to-use*
Stabilizer	contains 0.1 % ProClin®300
Storage	–20 °C or 2–8 °C
Stability	at –20°C: 1 year, repeated freeze/thaw cycles possible at 2–8°C: 6 months

Instructions for use

The newly developed *CrossDown* Buffer lowers cross reactivities, unspecific binding and matrix effects in immunoassays like ELISA, EIA, Western blotting, immuno-PCR, protein arrays, multianalyte immunoassays and immunohistochemistry – depending on the characteristics of the assay type and the used antibodies.

Mix the buffer thoroughly immediately before use. *CrossDown* Buffer is used instead of a sample buffer or antibody dilution buffer for the immunological reaction. *CrossDown* Buffer is not suitable for blocking of surfaces. For blocking of surfaces we recommend *Blocking Buffer I* (Order No. A7099). *CrossDown* Buffer is not suited as a sample buffer for electrophoresis.

Examples of use

ELISA dilution buffer for specimen and for the detection antibodies
Western blotting dilution buffer for primary and secondary antibodies
Immunohistochemistry dilution buffer for primary and secondary antibodies
Protein arrays dilution buffer for specimen and for the detection antibodies

Dilution of the specimen Standards and specimen for ELISA and protein arrays can be diluted with *CrossDown* Buffer at 1:2 or higher. Standards and specimen should be treated strictly the same way.

Dilution of antibodies Antibodies can be diluted with *CrossDown* Buffer in a user-defined manner, depending on the recommendation of the data sheet of the antibodies. This is the same for primary and secondary antibodies.

Appearance of signal reduction In some cases a smooth reduction of the wanted signal can be observed. *CrossDown* Buffer reduces low- and middle-affinity binding. That means that by the use of low- and middle-affinity antibodies or polyclonal antibodies a smooth reduction of signals can appear. Polyclonal antibodies normally contain low- and middle-affinity binding components.

In the case of polyclonal antibodies a moderate increase of the concentration of the antibody can lead to the previously seen signals. Unwanted low- and middle-affinity binding will be still reduced by *CrossDown* Buffer.

In the case of low- and middle affinity antibodies (also monoclonal antibodies) a pre-dilution of *CrossDown Buffer* with salt-free water can be useful to get the previously seen signal. But in this case also the unwanted bindings or cross-reactivities can partly occur again, depending on the chosen dilution with water.

Although *CrossDown* Buffer is used as an assay buffer it is necessary to saturate surfaces like ELISA-wells or membranes with a blocking agent. We recommend the use of *Blocking Buffer I* (Order No. A7099). *CrossDown* Buffer can be used additionally as a washing buffer – especially in delicate or interference-sensitive assays like immuno-PCR. Components of immunoassays – as well as of *CrossDown* Buffer– may quench the fluorescence of fluorescein dyes. Therefore we recommend the use of Oyster*- (Denovo Biolabels), CyDye**- (Amersham) or Alexa***- (Molecular Probes) fluorescence dyes.

We strongly recommend to test the effectiveness of *CrossDown* Buffer for a certain application.

CrossDown in FACS analysis *CrossDown* buffer can replace the normally used FACS analysis assay buffer and is applied like the original assay buffer. In case that *CrossDown* is to "active" (i.e. reduction of the specific signal too), the most convenient way is to dilute the buffer with the original assay buffer (dilution 1 : 2 to 1 : 10). Alternatively, physiological buffers like PBS or Hepes can be used for diluting *CrossDown*.

*Oyster is a registered trade mark of the company Denovo Biolabels.
**CyDye is a registered trade mark of the company Amersham Biosciences.
***Alexa Fluor Dye is a registered trade mark of the company Molecular Probes.

Literatur

[1] *Miller, J.J. (2004) Clinical Laboratory International* **28**, *(2), 14-17*
[2] *Kusnezow, W., Hoheisel, J.D. (2003) J. Mol. Recognit.* **16**, *165-176*
[3] *Patton, W.F. (2000) Electrophoresis* **21**, *1123-1144*
[4] *MacBeath, G. (2002) Nat. Genet. 32, 526-532*
[5] *Miller, J.J., Valdes, R.Jr. (1992) J Clin Immunoassays* **15**, *97-107*
[6] *Wood, W.G. (1991) Scand. J. Clin. Lab. Invest. Suppl.* **205**, *105-112*
[7] *Kricka, L.J. (1999) Clinical Chemistry* **45** *(7), 942-956*
[8] *Span, P.N., Grebenchtchikov N., Geurts-Moespot, J., Sweep, C.G.J. (2003) Clinical Chemistry* **49** *(10), 1708-1709*

Frequently Asked Questions about CrossDown Buffer

What's so special about CrossDown?

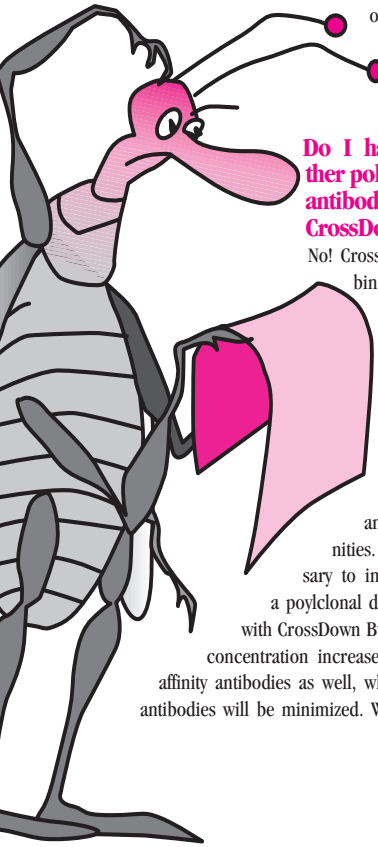
The newly developed CrossDown Buffer lowers cross reactivities, unspecific binding and matrix effects in immunoassays like ELISA, EIA, Western blotting, immuno-PCR, protein arrays, multianalyte immunoassays and immunohistochemistry. The specific, high affinity binding of antibody to analyte stays, while unwanted binding of the antibody is prevented.

How do I use CrossDown Buffer?

CrossDown Buffer is used instead of sample buffer or antibody dilution buffer for the immunological reaction. The sample (e.g. human serum or plasma) and the detection antibody are diluted with CrossDown Buffer, depending on the source of the interference. Assuming nonspecific binding or cross reactivity of the detection antibody, dilution with CrossDown Buffer is recommended.

Does CrossDown Buffer replace a blocking buffer for surfaces (e.g. Western blot membranes or ELISA plates)?

No! CrossDown Buffer is not suitable for blocking of surfaces, but used for dilution of the sample and/or assay antibodies instead. For blocking of surfaces we recommend Blocking Buffer I (Order No. A7099).



Do I have to consider whether polyclonal or monoclonal antibodies are diluted with CrossDown?

No! CrossDown can be used in combination with both types of antibody preparations. In case of polyclonal antibodies, you have to keep in mind that CrossDown only supports high affinity binding. Low affinity binding is suppressed. Polyclonal antibodies are a mixture of antibodies with different affinities. Therefore it may be necessary to increase the concentration of a poylclonal detection antibody when used with CrossDown Buffer. Increasing the antibody concentration increases the concentration of high affinity antibodies as well, while the effect of low affinity antibodies will be minimized. Whether the concentration of an antibody in an assay has to be adjusted very much depends on the quality of the antibody

Why does CrossDown Buffer assist in reducing matrix effects?

The primary cause of the so-called matrix effect is based on unwanted, low-affinity binding of matrix components (e.g. serum proteins in samples from human serum) to analytes or antibodies. The analyte may be masked by proteins or other components of the sample matrix, preventing the binding of the antibody to its target. CrossDown Buffer prevents masking and support binding of antibody and analyte.
Antibodies may be masked by matrix components as well. CrossDown prevents this masking and reduces masking already present.

May I freeze-thaw the buffer several times?

The buffer can be frozen and thawed several times without a problem. After thawing, the buffer has to be mixed thoroughly before application to guarantee uniform distribution of the components.

Is the buffer used diluted or undiluted?

CrossDown Buffer is a *ready-to-use* solution. The sample or the detection antibody can be diluted directly in CrossDown Buffer.
In competitive assays and some applications in immunohistochemistry, it may make sense to dilute CrossDown Buffer with water or physiological buffer. The optimal conditions and ratios should be determined on a case by case basis.

Do buffer components influence color reactions?

The enzymatic activity of alkaline phosphatase or peroxidase are not negatively influenced by CrossDown Buffer. In fact, the opposite is the case. Users report an increased enzyme activity, when labeled detection antibodies were incubated with CrossDown Buffer.

Can CrossDown be applied together with fluorescent dyes, e.g. in protein chip applications?

Yes. There are positive results in protein chip applications. In some applications using a few cyan-dyes (e.g. Cy5, Cy3, oyster dyes), an increased signal was observed.

Why?

using CrossDown Buffer??

	CrossDown Buffer	Antibody Diluent	HAMA-Blocker
Interference effects	**Minimisation of interference** – regardless whether cross-reactivities, matrix effects or unspecific binding of assay components	No minimisation of interference	Minimisation only of interference derived from HAMAs – (Human Anti Mouse Antibodies) – All other interference effects lead to wrong results!
Background	**Minimisation of background**	No effects	Effects only if background comes from HAMAs
Quality of results	**Increase in reliability** guarantees better results by avoiding interference	No positive effects on reliability	Increase in reliability only when specimen / samples include HAMAs
Usability	For use in **all immunoassays**	Some products only for use in ELISA or Western blotting, many different specialised products	For use only for human specimen/ samples
Usability with different detection methods	**Usable with all common detection methods,** very good results with peroxidases, phosphatases and fluorescent labels	Usability depends on product, some only for use with peroxidases others only for use with phosphatases, negative quenching with fluorescent dyes has to be checked with some products	Usability depends on product, some only for use with peroxidases others only for use with phosphatases, negative quenching with fluorescent dyes has to be checked with some products
Ease of application	***Ready-to-use***	Some products recommend pre-dilutions with other buffers	Some products recommend pre-dilutions with other buffers
Stabilisation of antibodies	**Assay antibodies are stabilised** in *CrossDown* Buffer, even storage of antibodies in *CrossDown* is possible	No effects on stability of assay antibodies	No effects on stability of assay antibodies
Effects on validation (e.g. for new FDA-guidance for industry)	**Positive effects, variations decrease**, false results are avoided, validations can be passed successful and easy	No positive effects on validation, interference like matrix effects or cross-reactivities lead to high variations or false results	Positive effects only if HAMAs are inside the samples. Then false results can be avoided. No effect on matrix effects and other cross-reactivities
Storage and transportation	Cooling and freezing for long-time storage, repeated freezing and thawing possible, but **no cooled transportation needed**	Cooling or freezing for long-time storage, most products recommend cooled transportation	Cooling or freezing for most products necessary, cooled transportation needed

Sample Buffer T- A7101

Sample buffer and dilution buffer for antibodies for use in immunoassays and immunohistochemistry

A7101,0050	50 ml
A7101,0125	125 ml
A7101,0500	500 ml

Composition	detergent-free, phosphate-free
pH value	pH 7.2 ± 0.2
Preservative	contains 0.1 % ProClin®300
Storage	–20 °C or at 2–8 °C; repeated freezing and thawing cycles possible
Stability	at –20 °C: 1 year ; at 2–8 °C: 6 months

Instructions for use

Sample Buffer T- is a dilution buffer for specimen and antibodies für direct use in immunoassays. Antibodies, coupled to alkaline phosphatase or peroxidase, can be diluted and used in *Sample Buffer T-* without problems, because *Sample Buffer T-* is free of phosphate.
Sample Buffer T- without detergents contains neither Tween® nor other detergents. Thus it is for use especially in **immunohistochemistry**, where detergents sometimes can make problems in detection.

Immediately before use the buffer should be mixed thoroughly. *Sample Buffer T-* is *ready-to-use*. Repeated freezing and thawing is possible. Primary and secondary antibodies can be diluted directly in *Sample Buffer T-*.

In case you observe increased background or unwanted matrix effects in the presence of *Sample Buffer T-*, we recommend the use of *CrossDown Buffer* (Artikel-Nr. A6485) instead of *Sample Buffer T-*.

Sample Buffer T+ A7134

Sample buffer and dilution buffer for antibodies for use in immunoassays

A7134,0050	50 ml
A7134,0125	125 ml
A7134,0500	500 ml

Composition	contains Tween®
pH value	pH 7.2 ± 0.2
Preservative	contains 0.1 % ProClin®300
Storage	–20 °C or at 2–8 °C; repeated freezing and thawing cycles possible
Stability	at –20 °C: 1 year ; at 2–8 °C: 6 months

Instructions for use

Sample Buffer T+ is a dilution buffer for specimen and antibodies für direct use in immunoassays. Antibodies, coupled to alkaline phosphatase or peroxidase, can be diluted and used in *Sample Buffer T+* without problems, because *Sample Buffer T+* is free of phosphate.

Sample Buffer T+ can be used for ELISA, EIA or Western blotting as well as for immuno-PCR or protein arrays. *Sample Buffer T+* is not for use as an electrophoresis buffer.

Immediately before use the buffer should be mixed thoroughly.
Sample Buffer T+ is *ready-to-use*. Repeated freezing and thawing is possible. Specimen with the analyte - as well as the detecttion antibody - is diluted in *Sample Buffer T+* and then used in the assay.
It is necessary to treat standards and specimen in the same way!

In case you observe increased background or unwanted matrix effects in the presence of *Sample Buffer T+*, we recommend the use of *CrossDown Buffer* (Artikel-Nr. A6485) instead of *Sample Buffer T+*.

Stripping Buffer I — A7140

Stripping buffer for Western blots for multiple reprobing

A7140,0050	50 ml
A7140,0125	125 ml

Composition	doesn't contain β-mercaptoethanol and DTT
pH value	pH 2.8 ± 0.2
Preservative	contains 0.1 % ProClin®300
Storage	–20 °C or at 2-8 °C
Stability	at –20 °C: 1 year at 2–8 °C: 6 months

Instructions for use

Ready-to-use

Stripping Buffer I removes reaction solution, primary and secondary antibodies from Western blotting membranes. After stripping the membrane can be used for a second detection (reprobing) with the same or other antibodies.

The membrane is incubated in a vessel with *Stripping Buffer I* for removing the antibodies. For this purpose gently shake the membrane in *Stripping Buffer I* for 30–60 minutes at room temperature.

After stripping, the membrane has to be washed with *Washing Buffer* and can be used for a second detection.
If you detect with alkaline phosphatase, the wash buffer should not contain phosphates.

Important: The designated incubation conditions are standard values, which have to be adopted by the user. Incubation times are depending on characteristics of the used antibodies.

Washing Buffer TrisT- (10X) — A7137

Washing buffer for use in ELISA, EIA, Western blotting and immunohistochemistry

A7137,0500	500 ml

Composition	Tris-based 10X buffer with 350 mM NaCl; contains no detergents
pH value	pH 7.2 ± 0.2
Preservative	contains 0.1 % ProClin®300
Storage	–20 °C oder bei 2–8°C Use working solution immediately!
Stability	at –20 °C: 1 year at 2–8 °C: 6 months

Instructions for use

This *Washing Buffer TrisT-* is made especially for immunohistochemistry. It should be used, whenever detergents can make problems in detection.

Crystals of salt can precipitate during storage at 2–8 °C or after freezing. Therefore *Washing Buffer TrisT-* must be warmed up to room temperature and should be mixed thoroughly before preparing the working solution. This leads to dissolving of salt after shaking. The stock solution is diluted 1:10 with deionized water to get the working solution. Use the working solution the same day.

Washing Buffer TrisT+ (10X) A7158

Washing buffer for use in ELISA, EIA and Western blotting A7158,0500 500 ml

Composition	Tris-based 10X buffer; contains Tween® and 350 mM NaCl
pH value	pH 7.2 ± 0.2
Preservative	contains 0.1 % ProClin®300
Storage	bei –20 °C or at 2–8°C Use working solution immediately!
Stability	at –20 °C: 1 year at 2–8 °C: 6 months

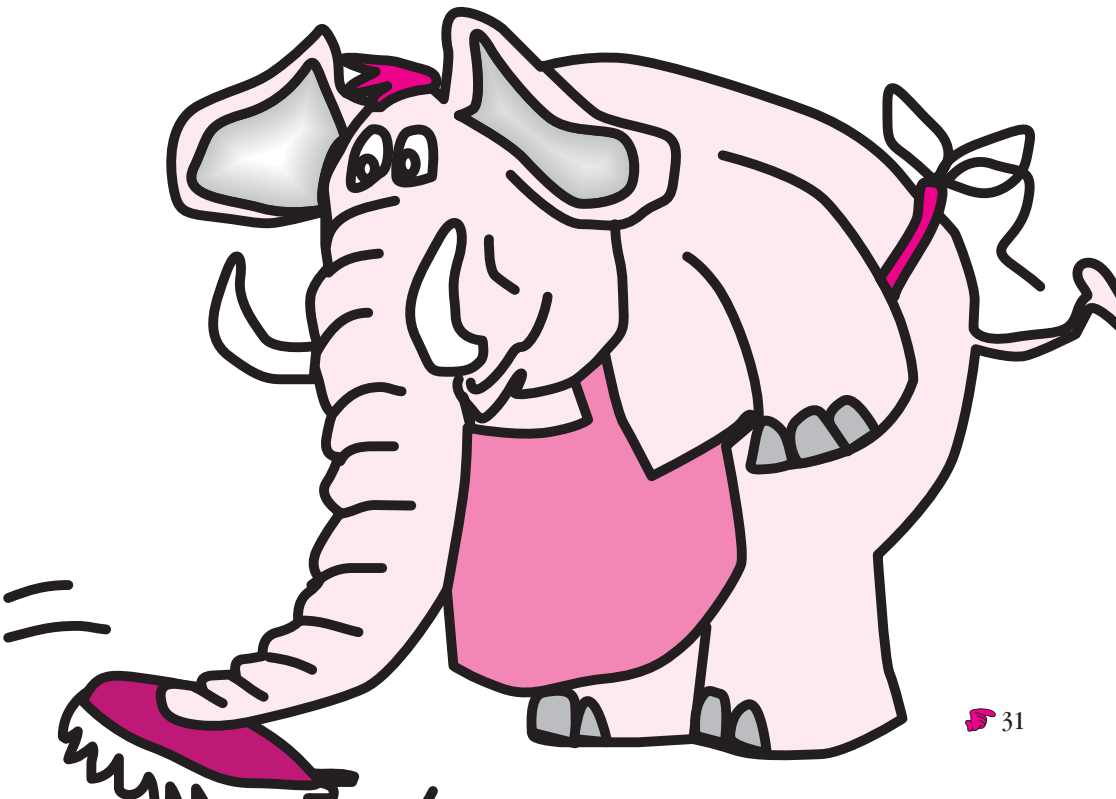
Instructions for use

Washing Buffer TrisT+ is used for many immunoassays. Washing steps are needed to remove unbound and excessive components, which are able to interfere with the assay. Application fields are ELISA, EIA, RIA, Western blotting as well as immuno-PCR, protein arrays and multianalyte immunoassays. It is applicable in automatic plate washers depending on salt tolerance of the washer.

Caution Make sure that you have checked the specifications and instructions of your washer!

Crystals of salt can precipitate during storage at 2–8 °C or after freezing. Therefore *Washing Buffer TrisT+* must be warmed up to room temperature and should be mixed thoroughly before preparing the working solution. This leads to dissolving of salt after shaking. The stock solution is diluted 1:10 with deionized water to get the working solution. Use the working solution the same day.

Especially for use in immunohistochemistry we offer *Washing Buffer TrisT-* without Tween or other detergents (Product No. A7137).



Related Products

Blocking Reagents	Prod. No.
Albumin acetylated	A0845
Albumin Fraction V (pH 7.0) Blotting grade	A6588
Denhardt's - Solution (50X) BioChemica	A2248
Denhardt's powder mixture (for 50X stock solution)	A3792
Dextran sulfate 500 sodium salt BioChemica	A2250
Dextran sulfate 500 sodium salt Molecular biology grade	A4970
Gelatin powdered pure Ph. Eur., NF	A1693
Heparin sodium salt	A3004
Nonfat dried milk powder	A0830
Polyvinylpyrrolidone (K90) Molecular biology grade	A2260
Salmon sperm DNA sodium salt	A2160
Salmon sperm DNA sodium salt (sonified)	A2159

Transfer membranes	Prod. No.
Reprobe Nitrocellulose supported 0.22 µm Transfer membrane	A5237
Reprobe Nitrocellulose supported 0.45 µm Transfer membrane	A5242
Pure Nitrocellulose unsupported 0.22 µm Transfer membrane	A5250
Pure Nitrocellulose unsupported 0.45 µm Transfer membrane	A5239
Pure Nylon Neutral Transfer membrane 0.22 µm (30 cm x 3 m)	A4399
Pure Nylon Neutral Transfer membrane 0.45 µm	A5248
Reprobe Nylon Positively charged Transfer membrane 0.45 µm (30 cm x 3 m)	A5255
PVDF-Star Transfer membrane 0.45 µm	A5243

Brochure Transfer Membranes

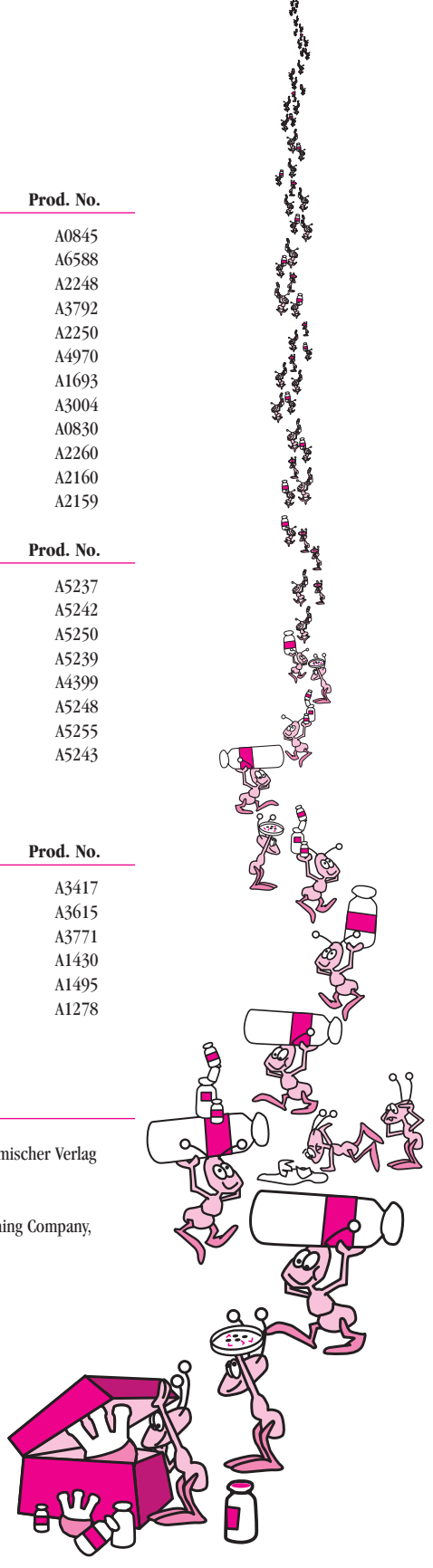
Other Related Products	Prod. No.
Chemiluminescence Detection Kit for Horseradish Peroxidase	A3417
Peroxidase fom horseradish EIA and Immunology Grade I	A3615
Peroxidase fom horseradish EIA and Immunology Grade II	A3771
Sodium azide pure	A1430
Streptavidin ultrapure	A1495
Thimerosal BioChemica	A1278

Further reading

Arnold M. Raem & Peter Rauch (Hrsg.) **Immunoassays** 2007 Elsevier Spektrum Akademischer Verlag (ISBN 3-8274-1636-1) german language

David Wild (Ed.): **The Immunoassay Handbook**. 3rd Edition. Elsevier Science Publishing Company, Amsterdam, Boston, Oxford 2005, ISBN 0-08-044526-8

Werner Luttmann, Kai Bratke, Michael Küpper, Daniel Myrtek: **Der Experimentator: Immunologie.** 2. Auflage. Spektrum Akademischer Verlag, Heidelberg 2006, (ISBN 3-8274-1730-9) german language



infotaining

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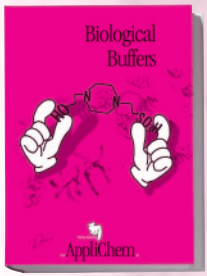
Gel Electrophoresis Size Marker

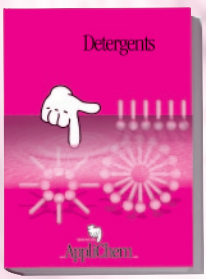
Ready-to-use DNA size marker and protein size marker for gel electrophoresis are available from many sources. Besides these standard products, AppliChem offers the full range of lyophilized DNA markers. The advantage of lyophilized marker is their outstanding stability. The long shelf life of more than 5 years is achieved by deproteinization and subsequent lyophilization. Read more about the usage of our markers in our new brochure.

Biological Buffers



Many biochemical processes are markedly impaired by even small changes in the concentration of free H^+ ions. It is therefore usually necessary to stabilise the H^+ concentration *in vitro* by adding a suitable buffer to the medium, without, however, affecting the functioning of the system under investigation.
Biological buffers are an essential part of each experiment. Several aspects have to be taken into account, when planning an experiment. AppliChem's brochure "Biological Buffers" imparts basic knowledge on the criteria for selecting the right buffer and requirements of buffers.

Detergents

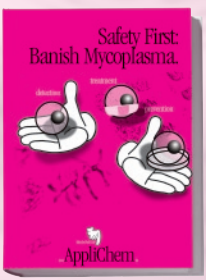


Detergents are more than just air bubbles. As diverse as the research objects and techniques are as diverse are the detergents available. The properties of e.g. SDS and octylglucoside are so different that you hardly may exchange them for the identical experiment.
Why do you apply SDS in gel electrophoresis but not dodecylmaltoside? Because your colleagues did it all the time before? AppliChem's brochure „Detergents" will help you selecting the best detergent for your assay.

Transfer Membranes

AppliChem supplies a range of transfer membranes designed and tested specifically for RNA, DNA and protein analysis. AppliChem also provides our customers with tried and proven protocols developed to obtain consistent reproducible and dependable results when used with AppliChem membranes.
22 protocols and all types of membranes – all from one source.

Safety First: Banish Mycoplasma



The contamination of cells with mycoplasma is a very common problem, even though it often goes unnoticed since no cloudiness appears in the cell culture. Mycoplasma are small and may pass the filtration units applied for preparing cell culture media. They do influence the growth, morphology and survival of the host cells, resulting in a strong influence on the test system and the results obtained. AppliChem offers a PCR-based test kit to test for infection, antibiotics for the treatment of infected cells, and cleansing reagents for CO_2-incubators and waterbaths to prevent infection.

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