THE APPROPRIATE BLOCKER – A SHORT OVERVIEW

Why do I have to block?

Free surfaces have to be blocked in immunoassays. This is true for many methods such as ELISA, protein arrays, immuno-PCR, Western blotting or immunohistochemistry.

The surfaces of ELISA plates or Western blotting membranes are optimized for efficient attachment of proteins and antibodies. This enables optimal assays with a high density of capture antibodies or capture molecules on the surfaces.

As a consequence of this optimization of the surfaces, many other proteins or peptides can attach on the surface. This attachment is reversible. Without blocking of surfaces, assay antibodies, tracers, analytes or other components of the sample would bind to the surface. This can lead to false results or high background signals. Direct attachment of detection antibodies to the ELISA well leads to a signal even in the absence of analyte. This has to be prevented. Blocking is necessary to saturate free binding capacities of the respective surfaces. This saturation can be achieved by covering the surface with a dense layer of molecules without gaps in this layer.

How to block?

After coating of the ELISA plate with the capture antibody (or antigen), the free spaces of the surface have to be blocked. A blocking solution contains a blocking agent in high concentration. Incubation is done in the microwell. This leads to an adsorption of the blocking agent to the free binding sites on the surface. Ideally a completely dense and gap-free layer is generated. Incubation time depends on the blocking agent and can be extremely short (in case of very good blockers). Generally it's recommended to use longer incubation times and to choose an appropriate operational procedure. Incubation times of 1–2 hours or overnight are common.

After blocking one can proceed with the assay. In addition to a blocking step a subsequent stabilisation step with Liquid Plate Sealer® is recommended. This can improve binding capacity of capture antibodies to the analyte, and can improve the results of some assays in terms of sensitivity and reproducibility. Additionally it's possible to store coated ELISA plates for longer periods before using them. This stabilizer is incubated in the well of the ELISA plate as with the blocking agent. Liquid Plate Sealer® covers the capture antibodies or coated antigens with a protective and easily soluble layer.

This layer has two effects. First, the layer can directly improve the structural conformation of many capture antibodies or antigens. The improvement in conformation occurs during incubation and following the drying process of the ELISA plate. This is demonstrated by a higher binding capacity of the capture antibodies compared to untreated capture antibodies. This effect can be measured directly after drying of the plate, when the effect is relevant to the coated capture molecules. The second effect is a long-term stabilization of the coated biomolecules. ELISA plates can be stored for long periods at 4°C after stabilization. In most cases operational procedures in the lab can be facilitated by coating and storing many plates in stock. Stabilizing can also be used to reduce coefficients of variations of ELISAs. The positive effects of Liquid Plate Sealer® strongly depend on the biomaterials which are used (antibodies and antigens). Therefore the positive effects cannot be quantified generally but only based on the respective assay.

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Which one is the perfect blocker?

There is no perfect blocker which is well suited to every assay. A blocker has to match the respective assay and has to be selected individually for any new assay. Due to optimized plates and western blotting membranes for protein or peptide binding, protein-based blockers are more effective than other blocking solutions. In most cases synthetic blockers are less efficient than blockers based on proteins or peptides.

The classic option: Blocking with BSA

BSA-Block is a classic blocker based on BSA (bovine serum albumine). BSA was one of the first proteins which was available in good purity and in appropriate amounts for research applications. For this reason it was used very early on as a blocking reagent in immunoassays. It has a proven track record of many assays in which it has been used. The product BSA-Block is produced in a certified company from purified BSA. As with all solutions based on BSA, the blocking effectiveness is not optimal. BSA is a protein, which is just too large to build a layer with optimal density. But for most assays it is acceptable and for some assays BSA is the optimal blocker. There are antibodies which show optimal binding efficiency only in the presence of BSA. Such antibodies, coated as capture antibodies, can show best results after blocking with BSA. For some antibodies BSA-Block will show better assay results than any other blocker because of a direct positive effect on the capture antibodies. Combined with a subsequent incubation with Liquid Plate Sealer® as a stabilizer for the coated antibodies, the positive effect of BSA on the antibodies can be improved significantly. The blocking effectiveness of BSA is excellent compared to synthetic blockers but lower than the effectiveness of modern blocking solutions.

The most efficient blocker

The objective in the development of many assays is good performance and reproducibility combined with good economics. For such assays, CANDOR has developed the peptide based blocker SmartBlock™. The lot-to-lot consistency is similar to the high consistency of synthetic blockers. SmartBlock™, as a peptide based blocker, can bind very well on surfaces which can also bind antibodies or proteins. Additionally, a chemical optimization of the peptides enhances binding on protein-binding surfaces. Therefore the effectiveness of blocking is higher compared to many other blockers. Nevertheless it's an economical product which can also be used in Western blotting and in the production of ELISA kits. For research assays and applications, SmartBlock™ is the most efficient blocker.

The most effective blocker

For many years, casein has been described in the literature as a suitable blocking protein. The blocking efficiency of casein is often better than of BSA. Casein is offered in many commercially available blocking reagents. It is common during production to dissolve and to filter the casein to remove undissolved components. CANDOR offers a casein based blocker with the product The Blocking Solution. However The Blocking Solution is not comparable to conventional casein blockers from other manufacturers. Highly purified casein is chemically modified in a proprietary production process developed by CANDOR. During production the modified casein becomes fragmented. The result is a solution with a widely and consistently spread spectrum of different sized fragments of modified casein. In spite of this difficult production process, the lot-to-lot consistency is extremely good. Every batch of The Blocking Solution will give the same reproducible results in assays. This allows the routine use in GLP labs or in production of modern diagnostics with extremely low detection limits e.g. diagnostics of modern biomarkers. The result of the proprietary production process is an extremely effective blocker.

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The very high blocking effectiveness compared to alternative blocking agents like BSA can be easily quantified with the measurement of the coefficient of variation. The extremely high effectiveness of The Blocking Solution is not generally necessary in every assay. We recommend using The Blocking Solution for critical applications which need an exact, reliable and reproducible quantification of analyte. The Blocking Solution is also recommended for assays with rare and precious specimens.

Are there any blockers which I shouldn't use?

We recommend that you do not use blockers which can strongly differ in their composition from lot-to-lot or which can be contaminated. With such blockers you can observe changing results or different background depending on the batch. Use of such blockers is not suitable for regulated areas like GLP labs or in the production of human diagnostics kits. In these areas a changing purity is not allowed and would implicate an extensive validation for every batch.

These kinds of improper blockers include blockers based on milk powder and some commercially available blockers e.g. based on fish extracts and many other animal based extracts. In the case of milk powder it should be mentioned that milk powder is just food and not a chemical for lab use. Milk powder is the cheapest possible blocker but it should only be used if variable or unclear results resulting from contamination are not critical. Reasonable applications for milk powder, as a cheap blocker, are assays in some research areas and for educational use of assays e.g. at universities.

For reliable results, the use of milk powder or of unpurified blocking extracts should be avoided. Blockers based on fish (or other animal) extracts can be suitable if the material's origin are standardized sources and if standardized production and purification methods apply. But this is a theoretical point of view due to the fact that most suppliers don't guarantee standardized resources, purification processes and good lot-to-lot consistency. Therefore it's difficult to find such blockers on the market.

In conclusion it is necessary to search and find for every assay an appropriate blocker. Before choosing an adequate blocker it has to be clear how important the quality of the assay or the quality of the results have to be. In case of precious specimens or the need for reliable results (e.g. regulatory requirements) one has to look after high blocking efficiency. In case of lower requirements on the quality of results one can also use simple blockers. Assays for education and training e.g. at universities or schools can be handled with milk powder without any problems.

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