CrossDown Buffer

Immunoassay buffer for minimisation of unspecific binding, cross-reactivity and matrix effects
Product No. A6485

Description

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.

pH-Value: pH 7.2 ± 0.2
Phosphat-free, *ready-to-use*

Stabilizer: contains 0.1 % ProClin® 300

Storage: -20°C

The recommended temperature for long term storage is -20°C. Repeated freeze/thaw cycles are possible without loss of function.
(CrossDown Buffer may be stored at 2-8°C. However, shelf life is reduced by 50% at this storage temperature.)

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

Instructions for use

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.

Applications

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-reactivity 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.

Literature

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PanReac AppliChem ITW Reagents

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	**Useable with all common detection methods**, very good results with peroxidases, phosphatases and fluorescent labels	Useability 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	Useability 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