EUROPE
LIEGE SCIENCE PARK • 4102 Seraing • BELGIUM • Tel.: +32 4 372 74 00
• Fax: +32 4 372 75 00 • Toll-free: + 800 666 00 123 • info@eurogentec.com
• www.eurogentec.com

NORTH AMERICA
ANASPEC – 34801 Campus Drive • Fremont, CA 94555 • USA
• Tel.: +1-510-791-9560 • Toll-free: +1 800-452-5530 • Fax: +1 510-791-9572
• service@anaspec.com • www.anaspec.com

Technical Data Sheet

iD Blocking Kit
ID-WCBLO1-001

Eurogentec products are sold for research or laboratory use only and are not to be administered to humans or used for medical diagnostics.

Description
The iD Blocking Kit is optimised for quick blocking of any membranes used for Dot blot and Western blot. It can also be used for quick blocking of any solid surface such as microtiter plates used for ELISA.
This kit enables the complete blocking of the solid surface in just 5 minutes. Furthermore, the iD blocking Kit significantly increases the Western detection sensitivity compared to the classical milk blocking. Lower amount of antibodies are needed to reach the same antigen detection level as using classical blocking method.
Please note that when the antibody has a similar affinity for its antigen than for the blocking reagent, the signal to noise ratio is too low to detect clearly the antigen.

The iD Blocking Kit is compatible with the Eurogentec's iD TMB Substrate (ID-SUTMB1-060) and iD HRP Substrate Kit (ID-SUHRP1-060).

Key Features
> Blocking in only 5 minutes
> Easy to perform
> Sensitive

Storage
The kit remains stable for 2 years when stored at 4°C.

Kit Content
The kit contains enough reagents for 20 blockings

Components	Volume
Pretreat A	2 x 50 mL
Pretreat B	2 x 50 mL

Protocol
Materials needed but not provided:
Wash solution: PBST or any wash solution that you regularly use is fine.
Western Blot Plates

Blocking procedure:
This procedure is optimized for one 7.5 x 8 cm (or 10 x 10 cm) sheet of membrane. Reagent volumes may be increased or decreased in proportion to the size of membrane used.

Just before the protein transfer from gel to membrane is completed, make the iD blocking solution by mixing 10 ml of pretreat A solution with 10 ml of pretreat B solution in a plate.

Place the membrane (no wash of the membrane after transferring is needed) in the iD blocking solution
Incubate on a shaker for 5 minutes at room temperature.

After incubation, rinse the membrane twice with 15 ml of wash solution (any wash solution that you regularly use is fine).

Continue and complete the Western blotting procedure as usual.

Using iD blocking Kit for ELISA:
The iD blocking kit can be used for quick blocking in ELISA after antigen coating. However, do not use the kit after primary antibody binding, because the kit reagents can remove the antibody from antigen.

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Troubleshooting

Problem	Probable cause	Solution
The signal is weak or invisible.	There is too little protein loaded.	Load more protein(s) onto the SDS-PAGE gel
	Poor transfer efficiency	Optimize the transfer time and /or the electrical current. Make sure that there are no air bubbles between the membrane and the gel.
	The primary antibody has a low affinity for the antigen.	Increase the incubation time of the membrane with primary antibody.
There is high background or non-specific bands on the blot	Too much primary antibody or secondary is used.	Reduce the amount of antibody.
	The wash time is too short.	Wash the membrane longer or add one or two more wash steps.
	The equipment or reagents have become contaminated.	Use a clean container for each rinse and wash step. Wear gloves and use clean forceps to handle membranes.

For further information please contact our Customer Help Desk:

For Europe:
E-mail: info@eurogentec.com
Tel: +32 4 372 76 65

For USA:
E-mail: service@anaspec.com
Tel: +1 510 791 9560