PRODUCT INFORMATION SHEET

STANDARD WESTERN-BLOT PROTOCOL

1. Perform SDS polyacrylamide gel electrophoresis (SDS-PAGE) with your sample of choice and transfer proteins to either a nitrocellulose or a PVDF membrane.

2. Block residual binding sites by incubating the membrane in *"blocking buffer"* with the appropriate detergent and 1-3% of **Perfect-Block** in either PBS (phosphate buffered saline) or TBS (Tris buffered saline) for 25-60 minutes at RT and constant agitation.

3. Incubate the membrane with the appropriate first antibody in freshly prepared **Perfect-Block** "*blocking buffer*" either at RT for several hours or overnight at 4°C with constant agitation.

4. After first antibody incubation, wash the membrane several times with *"blocking buffer"*

5. Incubate the membrane with secondary antibody of choice in "*blocking buffer*" for 1-2 hours at RT and constant agitation.

6. Wash membrane several times in *"blocking buffer"*

7. Wash membrane twice in PBS or TBS

8. Use detection method of choice

Perfect-Block was mostly used in Western blot application with enhanced chemiluminescence detection (ECL).

PRODUCT INFORMATION SHEET

Western-Blot with anti-Phosphotyrosine antibodies
(according to Kanakura et al., 1991; JBC (1), 490-495)

* After electrophoretical transfer of the proteins, residual binding sites on the membrane were blocked by incubating the nitrocellulose in TBST (10 mM Tris pH 8.0, 150 mM NaCl; 0.5% TWEEN 20) containing 3-5 % **Perfect-Block** ("*blocking solution*") for 20-30 minutes at RT.

* Wash 2 x 5 min in TBST (0,5% Tween; pH 8.0) and incubate for 1-2 hours at RT or overnight with anti-P-Tyr monoclonal antibodies in **Perfect-Block** "*blocking solution*" at 4°C.

* After incubation wash 4 x 10 minutes in TBST (0,5% TWEEN 20) under constant agitation.

* Re-block the blot for additional 10-15 minutes with fresh **Perfect-Block** "*blocking solution*".

* Incubation with secondary anti-mouse antibody (HRP-coupled) in **Perfect-Block** "*blocking solution*" for the desired time.

* Wash 2 x quickly and 3 x 15 minutes in TBST (0,5% TWEEN 20).

* Detection with enhanced chemiluminescence system.

Perfect-Block worked also well using anti-phosphoserine or anti-phosphothreonin antibodies. In the meantime, very good experience has been made also with glyco- and lipoproteins.

References:

- Fritsche, J. et al., MCN (Molecular Cellular Neuroscience) 14, 398-418; 1999
- used in Chen, M.S. et al., Nature Vol. 403, 434-439; 2000

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