

Protocol for utilisation of PAb to PARP-1 (Prod. No. ALX-210-221) in Western blot

50µl of PAb to PARP-1 (Prod. No. ALX-210-221) is enough to make 25 Western blots from minigels, without considering that the diluted antibody can be re-used many times*.

Conditions for detection of PARP or apoptosis-cleaved PARP by Western blot from cell culture:

The number of cells detectable by ALX-210-221 during Western blot experiment depends on the cell line; 50'000 to 100'000 cells/well are generally sufficient.

After treatment of the cells (2 plates), determination of the number of cells is one in one plate by classical trypsinisation procedure; in the second plate, cells are scraped with a rubber policeman in sterile cold PBS (pH 7.4) or cold isotone [or spinned in case of work on suspended cells such as HL-60], then spinned in cold PBS (pH 7.4) or cold isotone 10 min at 1'000 rpm at 4°C. The supernatant is removed and the pellet is resuspended in a defined volume of reducing loading buffer (62.5mM TRIS, pH 6.8, 6M urea, 10% glycerol, 2% SDS, 0.003% bromophenol blue, 5% 2-mercaptoethanol (freshly added, i.e. 50µl of stock solution (14.3mol/l) in 1ml loading buffer)). Resuspension of cells in loading buffer can be done with a P1000 pipetman because the solution will be very viscous due to lysis of cells and hence presence of DNA. To break DNA, a sonication on ice is done for 20 sec. (microtips at limit, 40% duty cycle, Sonicator Vibracell, Sonics and Materials, CT). [Omission of sonication and urea in sample buffer will result in incomplete PARP extraction and solubilization]. Samples are now ready and either kept at -20°C before analysis or directly incubated 15 min at 65°C before loading on a SDS-PAGE [It is better to heat just the necessary aliquot at 65°C for 15 min and keep the rest of the sample at -20°C].

Electrophoresis and transfer:

Samples are loaded on a SDS polyacrylamide gel (usually 8 or 10%) in an electrophoresis apparatus Bio-Rad containing running buffer (25mM TRIS, 192mM glycine, 0.1% SDS) at 100V (for 1 h 15min) or 200V (for 45 min).

Proteins are transferred onto nitrocellulose membrane (Hybond C from Amersham, for instance) in a transblot cell (Bio-Rad) at 4°C under stirring. The transfer buffer is 25mM TRIS, 192mM glycine, 20% methanol and electrotransfer is done either at ~100V for 1 hour or at 35V overnight.

After transfer, nitrocellulose membrane is stained with Ponceau S (0.1% Ponceau S (w/v) in 5% acetic acid (v/v)) for 1 min then washed with deionized water to see molecular weight markers and check protein profile of the samples. The nitrocellulose membrane is then washed with PBSMT solution (1x PBS, pH 7.4, 5% non-fat powdered milk, 0.1% Tween 20) for 5 min to remove Ponceau S staining and is submitted to Western blot analysis.

Western blotting (all steps are done at room temperature under gentle shaking)

- Nitrocellulose membrane is saturated in PBSMT solution for 1 hour.
- For one minigel, 2 μ l of the primary antibody ALX-210-221 is diluted in 10ml of PBSMT solution [final dilution: 1:5'000]
- After overnight incubation (in 1mM sodium azide) with ALX-210-221, nitrocellulose membrane is washed in 1x PBS containing 2M deionized urea and 0.05% Tween 20 (3 x 5-10 min), then 10 min in 1x PBS containing 0.1% Tween 20.

NOTE: Incubation with ALX-210-221 can also be carried out for 2 hours (minimum).

- The secondary antibody (anti-mouse IgG conjugated to peroxidase from Jackson Laboratories) is diluted 1:2'500 in PBSMT and incubated 30 min. The blot is washed again in PBSMT 2 x 10 min and 3 x 5 min.
- The nitrocellulose membrane is washed 30 min with PBS before using the chemiluminescence reagents. Our detection system by chemiluminescence is "Renaissance" or "Renaissance Plus" from Dupont.

Erasure of the blot and reprobing:

- After detection step by chemiluminescence, blot can be stored at 4°C for a long time in a Saran Wrap or erased and reprobed with the same first antibody (more diluted or more concentrated) or with another antibody as follows:
- Nitrocellulose membrane is washed with 1x PBS, pH 7.4, containing 0.1% Tween 20 (4 x 5 min), then incubated in stripping buffer (62.5mM TRIS-HCl, pH 6.8, 2% SDS, 100mM fresh β -mercaptoethanol) for 30 min at 65-70°C. The membrane is washed again in PBS containing 0.1% Tween 20 (6 x 5 min).
- After this step, it is possible to check the absence of chemiluminescent signal (i.e. good stripping of antibodies from the membrane), by doing a new revelation with chemiluminescence reagents. An exposure of 5 min or more should not show any signal.
- The membrane can be washed again with PBS + 0.1% Tween 20 (4 x 5 min), then incubated in PBSMT solution for 1 hour and so ready for a new Western blot analysis.

*) The diluted primary antibody (ALX-210-221, 1:10'000) can be kept in PBSMT solution (1x PBS, 5% milk and 0.1% Tween 20) and is stable for many days (even a few weeks) at 4°C when containing 1mM sodium azide, 1U/ml penicillin and 1 μ g/ml streptomycin.

By reusing the diluted antibody, the background is even less (because of the progressive reduction of the antibody adsorption on the non-specific sites on the various blots).