

Anti-PARP

Cat. No. 11 835 238 001 100 µl (50 blots)

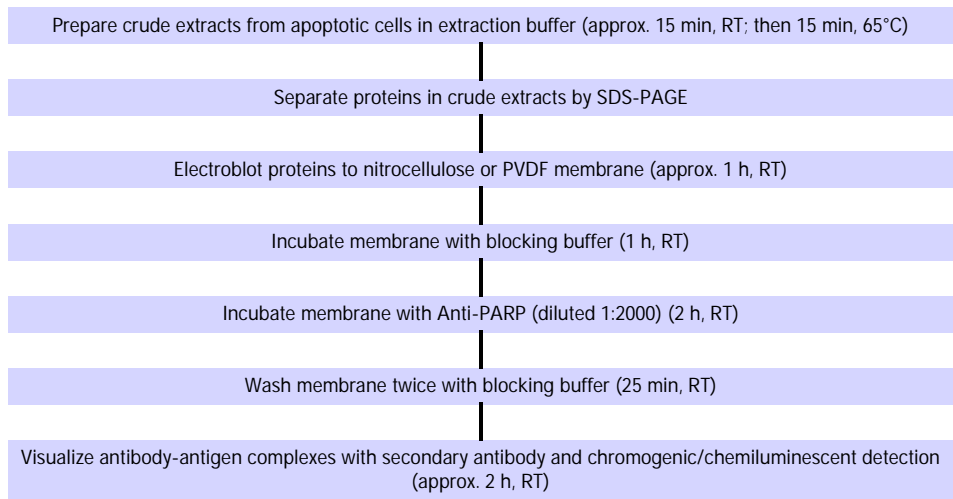
Type	Polyclonal antiserum, from rabbit
Useful for	Detection on Western blots of PARP cleaved by caspases during early stages of apoptosis
Samples	Crude cell extracts
Method	Western blot of apoptotic cell extracts, followed by indirect immunodetection of PARP cleavage fragment
Time	Approx. 5.5 h (immunodetection only)

Significance of reagent: Anti-PARP recognizes Poly-ADP-Ribose-Polymerase (PARP), a 113 kD protein that binds specifically at DNA strand breaks. PARP is also a substrate for certain caspases (for example, caspase 3 and 7) activated during early stages of apoptosis. These proteases cleave PARP to fragments of approximately 89 kD and 24 kD. Detection of the 89 kD PARP fragment with Anti-PARP thus serves as an early marker of apoptosis.

Test principle: The Anti-PARP antibody may be used to detect the 89 kD PARP fragment (and intact PARP) from apoptotic cell extracts on a Western blot. The procedure (Flow Chart 6) involves:

- 1 Preparing crude extracts of apoptotic cells (for instance, by sonication and incubation of 10^5 – 10^7 cells in the presence of urea, 2-mercaptoethanol, and SDS).
- 2 Separating proteins in the crude cell extracts on an SDS-polyacrylamide gel.
- 3 Transferring the separated proteins to a membrane by electroblotting.
- 4 Detecting PARP fragments (and intact PARP) on the membrane with the Anti-PARP antibody.
- 5 Visualizing the antibody-protein complexes with an enzyme-conjugated anti-rabbit IgG secondary antibody and a chromogenic or chemiluminescent enzyme substrate (see Table 3).





▲ Flow Chart 6: Assay of caspase activity with Anti-PARP.

Antibody supplied as: Polyclonal antiserum from rabbit, stabilized.

Sensitivity: PARP cleavage fragments from 3×10^5 apoptotic cells could be detected on a Western blot (Figure 19).

Specificity: On Western blots, Anti-PARP recognizes intact PARP from primates or rodents, as well as the large PARP fragment generated by caspases. Anti-PARP will immunoprecipitate intact PARP from primates or rodents.

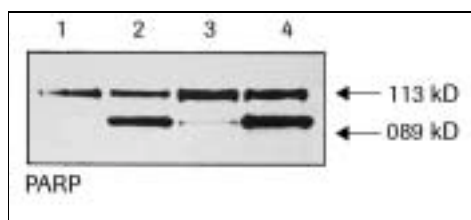
Can be used to assay:

- Crude cell extracts

Typical results: see Figure 19.

The appearance of a large (89 kD) cleavage fragment is indicative of caspase proteolytic activity.

Other applications: For more examples of how the Anti-PARP can be used in the lab, see Appendix, pages 133–134.



▲ **Figure 19: Detection of cleaved PARP in cell extracts of apoptotic CEM T cells.** CEM T cells were incubated with one of three apoptosis-inducing drugs. Cell extracts from 3×10^5 treated or untreated cells were fractionated on an 10% polyacrylamide gel in the presence of SDS. After electrophoresis, proteins on the gel were transferred to a PVDF membrane by electroblotting and the blot was blocked with 5% powdered milk. The blocked membrane was incubated with a 1:3000 dilution of Anti-PARP. Subsequent incubations with a peroxidase-conjugated anti-rabbit secondary antibody and a peroxidase substrate revealed the presence of PARP cleavage products on the blot. Note that the antibody recognizes both uncleaved PARP (113 kD) and the larger cleavage fragment (89 kD).

Lane 1: Untreated control cells

Lane 2: Cells treated with 100 ng/ml doxorubicin for 24 h

Lane 3: Cells treated with 1 mg/ml methotrexate for 24 h

Lane 4: Cells treated with 1 mg/ml cytarabin for 24 h.

(Data is courtesy of Dr. Ingrid Herr, German Cancer Research Institute, department of molecular oncology, Heidelberg, Germany)

Product	Cat. No.	Pack Size
BM Chromogenic Western Blotting Kit (Mouse/Rabbit)	11 647 644 001	for 2000 cm ² membrane
BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)	11 520 709 001	for 2000 cm ² membrane
BM Chemiluminescence Blotting Substrate (POD)	11 500 708 001 11 500 694 001	for 1000 cm ² membrane for 4000 cm ² membrane
CSPD (chemiluminescent AP substrate), ready-to-use	11 755 633 001	2 x 50 ml
CDP <i>Star</i> (chemiluminescent AP substrate) ready-to-use	11 685 627 001 11 759 051 001	1 ml 2 x 1 ml
BM Blue POD Substrate, precipitating	11 442 066 001	100 ml
BM Purple AP Substrate, precipitating	11 442 074 001	100 ml

▲ Table 3: Related products for visualization of Anti-PARP.

