

Technical Data Sheet

Purified Mouse Anti-Human PARP

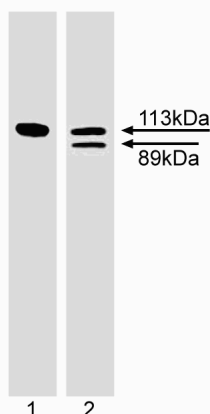
Product Information

Material Number:	556494
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	4C10-5
Immunogen:	Human PARP
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Target MW:	113 kD & 89 kD
Storage Buffer:	Aqueous buffered solution containing $\leq 0.09\%$ sodium azide.

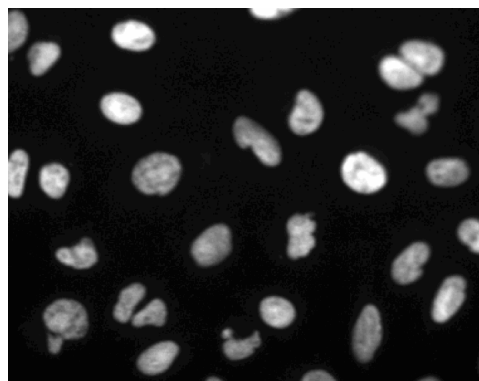
Description

PARP [Poly(ADP-ribose) polymerase] is a 113 kDa nuclear chromatin-associated enzyme that catalyzes the transfer of ADP-ribose units from NAD⁺ to a variety of nuclear proteins including topoisomerases, histones, and PARP itself. The catalytic activity of PARP is increased in non-apoptotic cells following DNA damage, and PARP is thought to play an important role in mediating the normal cellular response to DNA damage. Additionally, PARP is a target of the caspase protease activity associated with apoptosis. During apoptosis, PARP is cleaved from a 113 kDa intact form into smaller 89 kDa and 24 kDa fragments. This process separates the amino-terminal DNA-binding domain of the enzyme from the carboxy-terminal catalytic domain resulting in the loss of normal PARP function. Although the role of PARP in apoptosis remains to be elucidated, PARP cleavage is considered to be a marker of apoptosis. The 4C10-5 antibody recognizes both the intact 113 kDa form and 89 kDa fragment of PARP.

The 4C10-5 antibody has been reported to recognize both native and denatured PARP. Purified human PARP was used as the immunogen and the antibody reported to react with an epitope located in the NAD binding domain. In dot blot assays, the antibody reacts with the native enzyme in the presence or absence of bound DNA as well as after synthesis of covalently linked poly (ADP-ribose). The 4C10-5 antibody is routinely tested by western blot analysis of untreated Jurkat T cells and Jurkat T cells induced to undergo apoptosis.



Western blot analysis of PARP cleavage. Jurkat cells were untreated (lane 1) or induced to undergo Fas mediated apoptosis by treatment with anti-human Fas mAb, clone DX2 (cat. No. 555670) and Protein G for 4 hr (lane 2) and probed with the PARP antibody at 1-2 μ g/ml (clone 4C10-5). The 113 kDa intact form of PARP is seen in both the untreated and Fas mAb-treated cell lysates. However, the 89 kDa PARP cleavage fragment is only seen in the treated cell lysates.



Immunofluorescent staining of U-2 OS (ATCC HTB-96) cells. Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at $\sim 10,000$ cells per well. After overnight incubation, cells were stained using the Perm Buffer III protocol and the anti-PARP antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and HeLa (ATCC CCL-2) cells using both the Triton™ X-100 and Perm Buffer III protocols (see Recommended Assay Procedure).

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development
Flow cytometry	Reported
Immunoprecipitation	Reported
Dot Blot	Reported

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III or Triton™ X-100:
 - a. Add 100 µl of -20°C Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.OR
 - b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritified_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
550959	Jurkat Apoptotic Lysate Set I	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Triton is a trademark of the Dow Chemical Company.

References

Patel T, Gores GJ, Kaufmann SH. The role of proteases during apoptosis. *FASEB J.* 1996; 10(5):587-597. (Biology)
Ranjit GB, Cheng MF, Mackay W, Whitacre CM, Berger JS, Berger NA. Poly(adenosine diphosphoribose) polymerase in peripheral blood leukocytes from normal donors and patients with malignancies. *Clin Cancer Res.* 1995; 1(2):223-234. (Clone-specific: Flow cytometry, Western blot)