Technical Data Sheet

Purified Mouse Anti-Cleaved PARP (Asp214)

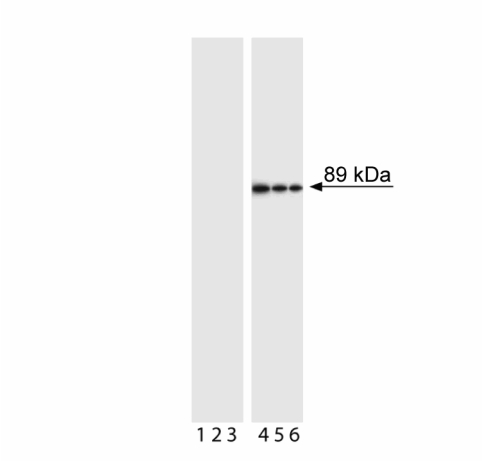
Product Information

Material Number:	**552597**
Size:	150 µg
Reactivity:	QC Testing: Human
Component:	**51-9000017**
Description:	Purified Mouse Anti-Cleaved PARP (Asp214)
Size:	50 µg (3 ea)
Concentration:	0.5 mg/ml
Clone Name:	F21-852
Immunogen:	Human cleaved PARP
Isotype:	Mouse IgG1, κ
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.
Component:	**51-16606N**
Description:	Camptothecin Treated Jurkat Lysate
Size:	50 µg (1 ea)
Storage Buffer:	SDS-PAGE buffer (62mM Tris pH 6.8, 2% SDS, 0.9% b-mercaptoethanol, 0.003% bromophenol blue, 5% glycerol)

Description

PARP (*P*oly [*A*DP-*R*ibose] *P*olymerase) 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 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. The PARP protein consists of an N-terminal DNA-binding domain (DBD) and a C-terminal catalytic domain separated by a central automodification domain. During apoptosis, Caspase-3 cleaves PARP at a recognition site (Asp Glu Val Asp Gly) in the DBD to form 24- and 89-kDa fragments. This process separates the DBD (which is mostly in the 24-kDa fragment) from the catalytic domain (in the 89-kDa fragment) of the enzyme, resulting in the loss of normal PARP function. It has been proposed that inactivation of PARP directs DNA-damaged cells to undergo apoptosis rather than necrotic degradation, and the presence of the 89-kDa PARP cleavage fraction is considered to be a marker of apoptosis.

A peptide corresponding to the N-terminus of the cleavage site (Asp 214) of human PARP was used as the immunogen. The F21-852 monoclonal antibody reacts only with the 89-kDa fragment of human PARP-1 that is downstream of the Caspase-3 cleavage site (Asp214) and contains the automodification and catalytic domains. It does not react with intact human PARP-1. Cross-reactivity with other members of the PARP superfamily is unknown. It may also recognize cleaved PARP in a number of other species due to the conserved nature of the molecule, although this has not been tested at BD Biosciences Pharmingen.



Western blot analysis of PARP (cleavage site-specific). *Jurkat cells were either left untreated (lanes 1-3) or treated with camptothecin (4 µM, 4 hours) to induce apoptosis (lanes 4-6). Lysates were probed with anti-PARP (clone F21-852, Cat. No. 552597) at concentrations of 0.25 (lanes 1, 4), 0.125 (lanes 2, 5), and 0.06 µg/ml (lanes 3, 6). Cleaved PARP is identified as a band of ~89 kDa in only the treated cells.*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store the antibody, component No. 51-9000017, at 4°C.
Store the lysate, component N,o. 51-16606N, at -20°C.

Application Notes

Application

Western blot	Routinely Tested
Intracellular staining (flow cytometry)	Tested During Development
Immunoprecipitation	Tested During Development

Recommended Assay Procedure:

Camptothecin treated Jurkat lysate [50 µg (1 µg/µl)] is provided as a positive control (51-16606N; store lysate at -20°C). Additional Jurkat lysate is available untreated (Cat. No. 611451) or as a set containing both untreated and campotothecin treated lysates (Cat. No. 550959) as ready-to-use western blot controls. Additional applications which are not not routinely tested at BD Biosciences Pharmingen include immunoprecipitation (2 µg/300 µg of lysates) and flow cytometry. The directly conjugated formats of the clone are recommended for flow cytometry. (Cat. No. 552933, and please inquire.)

Suggested Companion Products

Catalog Number	Name	Size	Clone
611451	Jurkat Cell Lysate	500 µg	(none)
550959	Jurkat Apoptotic Lysate Set I	500 µg	(none)
552933	PE Mouse Anti-Cleaved PARP (Asp214)	100 tests	F21-852
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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