

Technical Data Sheet

PE Mouse Anti-Cleaved PARP (Asp214)

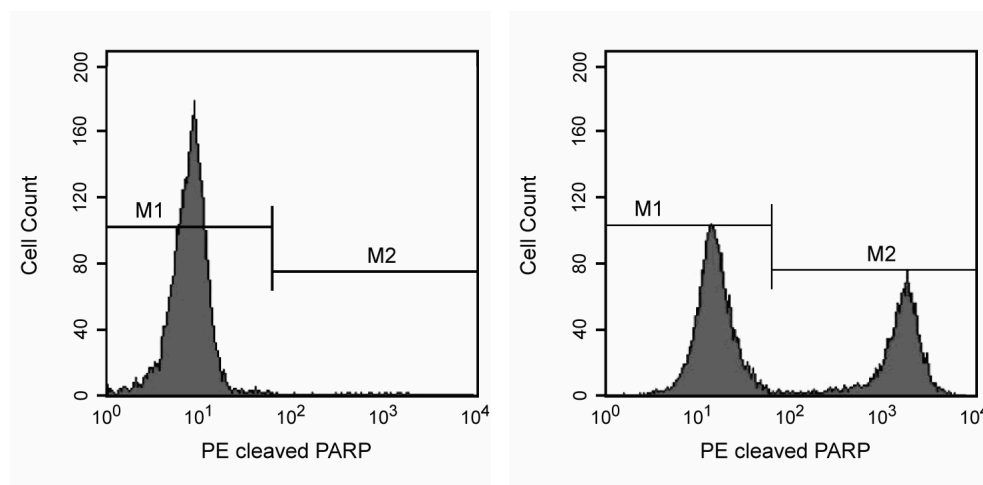
Product Information

Material Number:	552933
Size:	100 tests
Vol. per Test:	20 µl
Clone:	F21-852
Immunogen:	Human cleaved PARP
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤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 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.



Analysis of cleaved PARP in activated human T leukemia cells. Jurkat cells were either treated with camptothecin (right panel) or untreated (left panel). The cells were fixed and permeabilized, then stained with PE Mouse anti-Cleaved PARP according to the protocol included in this data sheet. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography. The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed. Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Recommended Assay Procedure:

Camptothecin (an extract of the Chinese tree *Camptotheca acuminata*) is a potent inhibitor of topoisomerase I, a molecule required for DNA synthesis. Camptothecin has been shown to induce apoptosis in a dose dependent manner in vitro. Camptothecin is used at BD Biosciences Pharmingen as a general method for inducing apoptosis.

Materials

- 1.0 μ M stock solution of camptothecin (Sigma; Cat. No. C-9911) in DMSO.
- Jurkat cell line (ATCC TIB-152), proliferating, at 1×10^6 cells/ml.
- Either Cytofix/Cytoperm™ Fixation/Permeabilization Kit (Cat. No. 554714) or Cytofix/Cytoperm™ solution (Cat. No. 554722) plus Perm/Wash™ buffer (Cat. No. 554723).

Procedure

1. Add camptothecin (4-6 mM final concentration) per 1×10^6 proliferating Jurkat cells. If desired, a control aliquot of untreated cells should also be prepared.
2. Incubate the cells for 4 hours at 37°C.
3. Wash the cells (camptothecin-treated and control aliquots) twice with cold PBS; then resuspend them in Cytofix/Cytoperm™ solution at 2×10^6 cells/ml.
4. Incubate the cells for 20 minutes on ice.
5. Pellet the cells, and aspirate and discard the Cytofix/Cytoperm™ solution.
6. Wash the cells twice at room temperature with 0.5 ml Perm/Wash™ buffer per 1×10^6 cells, and discard the supernatants.
7. Resuspend the cells in Perm/Wash™ buffer at 10×10^6 /ml.
8. Aliquot test samples of 1×10^6 cells per 100- μ l test.
9. Add 20 μ l antibody per test, and incubate for 30 minutes at room temperature.
10. Wash each test in 1.0 ml Perm/Wash™ Buffer and discard the supernatant.
11. Resuspend each test in 0.5 ml Perm/Wash™ Buffer and analyze by flow cytometry.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554723	Perm/Wash Buffer	100 ml	(none)
554714	BD Cytofix/Cytoperm™ Fixation/Permeabilization Kit	250 tests	(none)
554722	Fixation and Permeabilization Solution	125 ml	(none)
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1×10^6 cells in a 100- μ l experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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