Technical Data Sheet

Alexa Fluor® 700 Mouse Anti-Cleaved PARP (Asp 214)

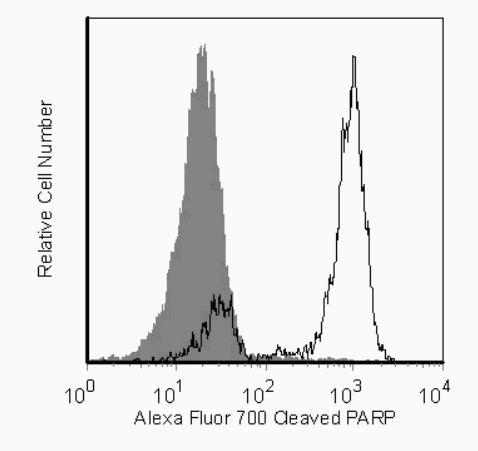
Product Information

Material Number:	**560640**
Size:	50 tests
Vol. per Test:	5 µl
Clone:	F21-852
Immunogen:	Human cleaved PARP
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

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.



Flow cytometric analysis of cleaved PARP in camptothecin treated Jurkat cells. *Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were either untreated (shaded) or treated with 4-6 µM camptothecin (Sigma-Aldrich Cat. No. C-9911) (unshaded), fixed and permeabilized with BD Cytofix/Cytoperm™ (Cat. No. 554714) and subsequently stained with the Alexa Fluor® 700 Mouse Anti-Cleaved PARP antibody. Histograms were derived from gated events based on light scattering characteristics for Jurkat cells. Flow cytometry was performed on a BD™ LSR II flow cytometry system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

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

The antibody was conjugated to Alexa Fluor® 700 under optimum conditions, and unreacted Alexa Fluor® 700 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow cytometry: 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 reported to induce apoptosis in a dose dependent manner *in vitro*.

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Materials

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

Procedure

1. Add camptothecin (4-6 µM final concentration) per 1 x 10^6 proliferating Jurkat cells. If desired, a control aliquot of untreated cells should also be prepared.
2. Incubate the cells for 4-6 hours at 37°C.
3. Wash the cells (camptothecin-treated and control aliquots) twice with cold PBS; then resuspend them in BD Cytofix/Cytoperm™ solution at 2 x 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 x 10^6 cells, and discard the supernatants.
7. Resuspend the cells in Perm/Wash™ buffer at 10 x 10^6 /ml.
8. Aliquot test samples of 1 x 10^6 cells per 100-µl test.
9. Add 5 µl 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
557882	Alexa Fluor® 700 Mouse IgG1, κ Isotype Control	0.1 mg	MOPC-21
554714	BD Cytofix/Cytoperm™ Fixation/Permeablization Kit	250 tests	(none)
554722	Fixation and Permeabilization Solution	125 ml	(none)
554723	Perm/Wash Buffer	100 ml	(none)

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-µl experimental sample (a test).
2. An isotype control should be used at the same concentration as the antibody of interest.
3. Alexa Fluor® 700 has an adsorption maximum of ~700nm and a peak fluorescence emission of ~720nm. Before staining cells with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
4. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.
5. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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