BP7143F

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Polyclonal Antibody to PARP-1 (cleaved) - FITC

Alternate names: ADP-ribose, ADPRT, ADPRT1, PARP, PARP-1, PARP1 - poly polymerase family, PPOL, member 1, pADPRT-1

Catalog No.: BP7143F

Quantity: 100 Tests

Background: Poly (ADP-Ribose) Polymerase (PARP) is a 116 kDa nuclear protein which is strongly activated by DNA strand breaks. During apoptosis, ICE family members, such as caspase-3 and -7, cleave PARP to yield an 85 kDa and a 25 kDa fragment. PARP cleavage is considered to be one of the classical characteristics of apoptosis.

Host: Rabbit

Immunogen: Chemically synthesized peptide corresponding to N-terminus of cleavage site (214/215) of human PARP

Format: **State:** Liquid Ig fraction
Purification: Sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a peptide spanning the cleavage site to remove antibody that is reactive with full length PARP. The final product is generated by affinity chromatography using a peptide corresponding to the PARP cleavage site.
Buffer System: Phosphate buffered saline, pH 7.2, with bovine serum albumin, containing 0.09 % sodium azide as preservative
Label: FITC – Fluorescein isothiocyanate

Applications: Flow cytometry (10 µL per 10e6 cells).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody specifically recognizes the 85 kDa fragment of cleaved PARP and can be used as a marker for detecting apoptotic cells.
Species: Human, Bovine.
Other species not tested.

Storage: Store the antibody at 2 - 8 °C up to one month or (in aliquots) at -20 °C for longer. Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: Duriez, P. J. and G. M. Shah (1997) Cleavage of poly (ADP-ribose) polymerase: a sensitive parameter to study cell death. Biochem. Cell Biol. 75(4):337-349.
Germain, M. et al. (1999) Cleavage of automodified Poly (ADP-ribose) polymerase during apoptosis. Evidence for involvement of caspase-7. J. Biol. Chem. 274(40):28379-28384.
Kaufmann, S. H. et al. (1993) Specific proteolytic cleavage of poly (ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res. 53(17):3976-3985.
Tewari, M. et al. (1995) Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly (ADP-ribose) polymerase. Cell

81(5):801-809.
Kumar, A.P. et al. (2001) 2-Methoxyestradiol blocks cell-cycle progression at G(2)/M phase and inhibits growth of human prostate cancer cells. Mol. Carcinogenesis (3):111-124.

Protocols:

Suggested Protocol for Induction of Apoptosis:
Apoptosis was induced by incubating Jurkat cells for 1 hour with 0.125 µg/mL anti-CD95/FAS antibody. The anti-PARP (214/215) FITC conjugated antibody has been shown to detect apoptosis when cells are treated with 1 µM camptothecin for 6 hours.

Suggested Staining Protocol:
After induction of apoptosis, wash cells 2 times with PBS/1% FCS. Fix cells by resuspending in 1 mL IC FixTM for 10 minutes at 4ºC then wash with PBS/0.1% sodium azide/1% FCS. The fixed cells can be stored at 4ºC for up to 7 days prior to staining. Aliquot fixed cells to a density of 10e6 cells/tube and wash 2 times in 1 mL IC PermTM. Pellet cells at 300 x g for 5 minutes, aspirate supernatant and resuspend in 40 µL IC PermTM. Add 10 µL anti-PARP (214/215) FITC and incubate at 4ºC for 30 minutes. Pellet cells and wash 2 times with 1 mL IC PermTM. Wash cells once in 1 mL PBS before resuspending the cells in 0.5 mL PBS, pH 7.3, for flow cytometric analysis.

Notes on Intracellular Staining Protocol:
At least one of the following specificity controls is recommended: 1) pre-incubating conjugated antibody with excess competing peptide; or 2) pre-incubating conjugated antibody with recombinant cleaved PARP.
High background staining has been reported for intracellular staining procedure. Increasing the concentration of non-specific protein (i.e., BSA or normal mouse serum) in IC PermTM to 2% may reduce this background staining.
In some cell lines, using 2 x IC PermTM buffer may increase the separation of positive and negative signals.

Solutions Used for Intracellular Staining Protocol:
IC FixTM: 4% paraformaldehyde in 50 mM phosphate buffered saline, pH 7.3.
IC PermTM: 50 mM phosphate buffered saline, pH 7.3, 1% (v/v) fetal calf serum, 0.1% (w/v) sodium azide and 0.1% (w/v) saponin.

Pictures:

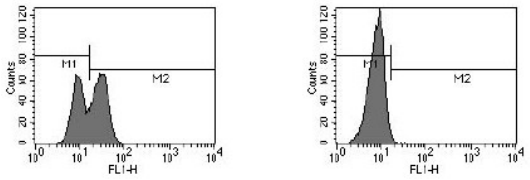

Figure: Apoptosis was induced as described in suggested protocol for apoptosis induction. The cells were fixed, permeabilized and stained with 10 µL/10e6 cells of the rabbit anti-PARP (214/215) FITC using the Staining Protocol (left-hand figure). In the right-hand figure, 10 µL of the anti-PARP (214/215) FITC conjugated antibody were pre-incubated with 0.2 µg/mL of the peptide corresponding to the PARP cleavage prior to addition to the apoptotic Jurkat cells. From the data, 54% of the cells were induced into apoptosis with the CD95/FAS antibody (clone 2R2).