# Rabbit (polyclonal) Anti-PARP Cleavage Site (214/215) Specific Antibody, FITC Conjugate

## PRODUCT ANALYSIS SHEET

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| **Catalog Number/Size:** | 44-699 (100 test size) |
| **Lot Number:** | See product label |
| **Quantity/Volume:** | 100 Tests/1 mL |
| **Form of Antibody:** | Fluorescein isothiocyanate conjugated, purified immunoglobulin in phosphate buffered saline, pH 7.2, with bovine serum albumin. |
| **Preservation:** | 0.1% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.) |
| **Purification:** | Purified from rabbit serum by 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. |
| **Immunogen:** | The antiserum was produced against a chemically synthesized peptide corresponding to N-terminus of cleavage site (214/215) of human PARP. |
| **Specificity:** | 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. This antibody specifically recognizes the 85 kDa fragment of cleaved PARP and can be used as a marker for detecting apoptotic cells. This antibody reacts with human and bovine cells. The reactivity with other species has not been determined. |
| **Suggested Working Dilutions:** | Use 10 µL per $10^6$ cells to detect cleaved PARP. The optimal antibody concentration should be determined for each specific application. |
| **Applications:** | This antibody is suitable for use in flow cytometry. The unconjugated antibody to cleaved PARP has been used successfully for Western blotting and immunohistochemistry applications. |
| **Storage:** | Store at 2-8°C for up to one month. For long term use, store in aliquots at −20°C. Avoid repeated freeze/thaw cycles. |
| **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. |



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**Suggested Staining Protocol:**

After induction of apoptosis, wash cells 2 times with PBS/1% FCS. Fix cells by resuspending in 1 mL IC Fix™ 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 $10^6$ cells/tube and wash 2 times in 1 mL IC Perm™. Pellet cells at 300 x g for 5 minutes, aspirate supernatant and resuspend in 40 µL IC Perm™. 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 Perm™. 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 Perm™ to 2% may reduce this background staining.

In some cell lines, using 2 x IC Perm™ buffer may increase the separation of positive and negative signals.

**Solutions Used for Intracellular Staining Protocol:**

**IC Fix™**: 4% paraformaldehyde in 50 mM phosphate buffered saline, pH 7.3 (cat. # FB001).

**IC Perm™**: 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 (cat. # PB001).

These buffers are also available as a convenient, ready to use kit (cat. # ANN0001).

**References:**


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 (cites the use of this antibody).


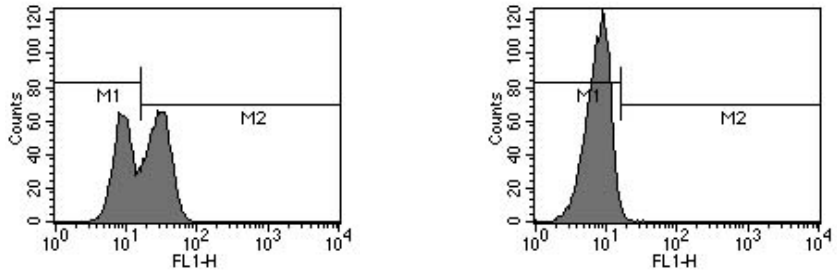


**Figure:** Apoptosis was induced as described in suggested protocol for apoptosis induction. The cells were fixed, permeabilized and stained with 10 µL/$10^6$ cells of the rabbit anti-PARP (214/215) FITC using the Staining Protocol (left-hand figure). In the right-hand figure, 10 µL of the anti-PARP (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 (AHS9552, clone 2R2).