Rabbit (polyclonal) Anti-PARP (214/215) Cleavage Site Specific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: 44698G (10 mini-blot size)

Lot Number: See product label

Volume: 100 µL

Form of Antibody: Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.

Preservative: 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)

Purification: Purified from rabbit serum by affinity chromatography using a peptide corresponding to the PARP cleavage site.

Immunogen: The antiserum was produced against a chemically synthesized peptide corresponding to the N-terminus of cleavage site (214/215) of human PARP.

Target Summary: Poly (ADP-Ribose) Polymerase (PARP) is a 116 kDa nuclear protein which is strongly activated by binding to DNA strand breaks. PARP plays a role in DNA repair as well as in other cellular processes, including DNA replication, cell proliferation and differentiation. 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.

Reactivity: Human PARP. Hamster, mouse and rat (86% homologous) PARP have not been tested

Applications: The antibody has been used in Western blotting. Previous lots have been used in immunostaining applications. Other applications may work but have not been tested.

Suggested Working Dilutions: For Western blotting applications, we recommend using the antibody at a 1:1000 starting dilution. The optimal antibody concentration should be determined empirically for each specific application.

Storage: Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

Expiration Date: Expires one year from date of receipt when stored as instructed.

Positive Controls Used: Jurkat or HeLa cells treated with staurosporine or etoposide (25 µM for 3 hours).

Related Products:

Antibodies:

STAT1 [pS^{727}], Cat. # 44382G

BAD [pS^{128}], Cat. # 44523G

p53 [pS^{392}], Cat. # 44640G

JNK1&2 [$pTpY^{183/185}$], Cat. # 44682G

p38 [$pTpY^{180/182}$], Cat. # 44684G

PKCα [pT^{638}], Cat. # 44962

PARP pan, Cat. # AHF0262

Stress Signal Sampler Pack, Cat. # 44648G

This product is for research use only. Not for use in diagnostic procedures.

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References:

Le Page, F., et al. (2003) Poly(ADP-ribose) polymerase-1 (PARP-1) is required in murine cell lines for base excision repair of oxidative DNA damage in the absence of DNA polymerase beta. J. Biol. Chem. 278(20):18471-18477.

Turturro, F., et al. (2002) Model of inhibition of the NPM-ALK kinase activity by herbimycin A. Clin. Cancer Res. 8(1):240-245 (cites the use of this antibody).

Leemans, J.C., et al. (2001) Depletion of alveolar macrophages exerts protective effects in pulmonary tuberculosis in mice. J. Immunol. 166(7):4604-4611 (cites the use of this antibody).

Soldani, C. and A.I. Scovassi (2002) Poly(ADP-ribose) polymerase-1 cleavage during apoptosis: an update. Apoptosis 7(4):321-328.

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.

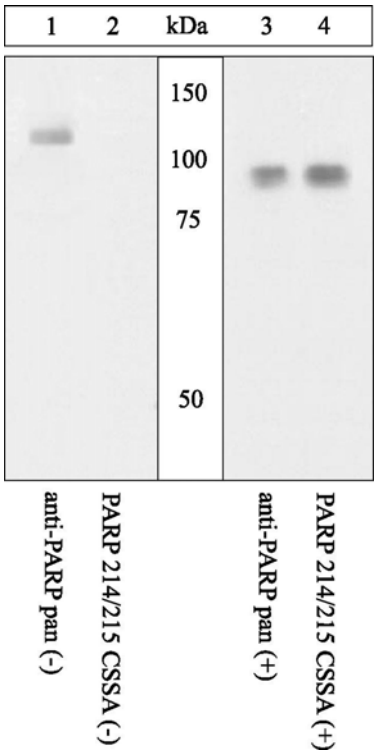

Figure 1. Western blot

Extracts of Jurkat cells untreated (1, 2) or stimulated with 0.5 µM staurosporine for 3 hours (3, 4) to induce apoptosis were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. The membrane was blocked with a 5% BSA-TBST buffer overnight at 4°C, then incubated with the anti-PARP pan (1, 3) or PARP (214/215) cleavage site-specific antibody (CSSA) (2, 4) for two hours at room temperature in a 3% BSA-TBST buffer. After washing, the membrane was incubated with goat $F(ab')_2$ anti-rabbit IgG HRP-conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that the PARP (214/215) CSSA only recognizes the 85 kDa fragment of PARP in apoptotic cells (4) and does not react with full length PARP (2), demonstrating the specificity of the antibody. The anti-PARP pan confirms that non-apoptotic cells express full length PARP of 116 kDa (1), which is then cleaved when apoptosis is induced (3).

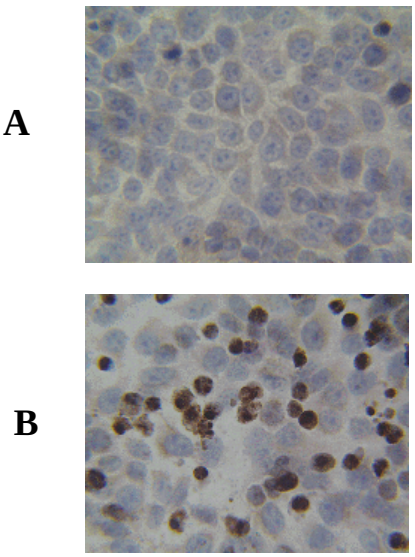

Figure 2. Immunohistochemistry

HeLa cells untreated (A) or induced into apoptosis with 0.5 µM staurosporine for 5 hours (B) and fixed in cold acetone for 5 minutes. Cells were incubated with the PARP (214/215) CSSA at 10 µg/mL. Cells were then incubated with biotinylated goat anti-rabbit IgG (Cat. # ALI4409) followed by ABC (Vector) and DAB. The data show that the PARP (214/215) CSSA specifically recognizes PARP in apoptotic cells. Taken together with Western blot results above, these data demonstrate the specificity of the antibody for cleaved PARP.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Invitrogen Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) overnight at 4°C or for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 starting dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for two hours at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer
Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer
Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline
Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer
Formulation:
100 mL Tris buffered saline
5 gm Ig-free BSA
0.1 mL Tween 20

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