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Anti-PARP/PARP1 Picoband™ Antibody (monoclonal, 2I2H4)

Catalog # ABO14997

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P09874
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-PARP/PARP1 Picoband™ Antibody (monoclonal, 2I2H4) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2 ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	142
Other Names	Poly [ADP-ribose] polymerase 1, PARP-1, 2.4.2.30, ADP-ribosyltransferase diphtheria toxin-like 1, ARTD1, DNA ADP-ribosyltransferase PARP1, 2.4.2.-, NAD(+) ADP-ribosyltransferase 1 {ECO:0000303|Ref.11}, ADPRT 1 {ECO:0000303|Ref.11}, Poly[ADP-ribose] synthase 1, Protein poly-ADP-ribosyltransferase PARP1, 2.4.2.-, Poly [ADP-ribose] polymerase 1, processed C-terminus, Poly [ADP-ribose] polymerase 1, 89-kDa form, Poly [ADP-ribose] polymerase 1, processed N-terminus, NT-PARP-1, Poly [ADP-ribose] polymerase 1, 24-kDa form, Poly [ADP-ribose] polymerase 1, 28-kDa form, PARP1 {ECO:0000303|PubMed:21680843, ECO:0000312|HGNC:HGNC:270}
Calculated MW	113084
Application Details	Western blot, 0.1-0.25 □g/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 2I2H4
Immunogen	E.coli-derived human PARP recombinant protein (Position: Q670-R858).

Human PARP shares 94% and 95% amino acid (aa) sequence identity with mouse and rat PARP, respectively.

Purification Immunogen affinity purified.

Storage Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name PARP1 {ECO:0000303|PubMed:21680843, ECO:0000312|HGNC:HGNC:270}

Function Poly-ADP-ribosyltransferase that mediates poly-ADP- ribosylation of proteins and plays a key role in DNA repair (PubMed:17177976, PubMed:18055453, PubMed:18172500, PubMed:19344625, PubMed:19661379, PubMed:20388712, PubMed:21680843, PubMed:22582261, PubMed:23230272, PubMed:25043379, PubMed:26344098, PubMed:26626479, PubMed:26626480, PubMed:30104678, PubMed:31796734, PubMed:32028527, PubMed:32241924, PubMed:32358582, PubMed:33186521, PubMed:34465625, PubMed:34737271). Mediates glutamate, aspartate, serine, histidine or tyrosine ADP-ribosylation of proteins: the ADP-D-ribosyl group of NAD(+) is transferred to the acceptor carboxyl group of target residues and further ADP-ribosyl groups are transferred to the 2'-position of the terminal adenosine moiety, building up a polymer with an average chain length of 20-30 units (PubMed:19764761, PubMed:25043379, PubMed:28190768, PubMed:29954836, PubMed:35393539, PubMed:7852410, PubMed:9315851). Serine ADP-ribosylation of proteins constitutes the primary form of ADP-ribosylation of proteins in response to DNA damage (PubMed:33186521, PubMed:34874266, PubMed:41062835). Specificity for the different amino acids is conferred by interacting factors, such as HPF1 and NMNAT1 (PubMed:28190768, PubMed:29954836, PubMed:32028527, PubMed:33186521, PubMed:33589610, PubMed:34625544, PubMed:34874266). Following interaction with HPF1, catalyzes serine ADP-ribosylation of target proteins; HPF1 confers serine specificity by completing the PARP1 active site (PubMed:28190768, PubMed:29954836, PubMed:32028527, PubMed:33186521, PubMed:33589610, PubMed:34625544, PubMed:34874266). Also catalyzes tyrosine ADP-ribosylation of target proteins following interaction with HPF1 (PubMed:29954836, PubMed:30257210). Following interaction with NMNAT1, catalyzes glutamate and aspartate ADP- ribosylation of target proteins; NMNAT1 confers glutamate and aspartate specificity (By similarity). PARP1 initiates the repair of DNA breaks: recognizes and binds DNA breaks within chromatin and recruits HPF1, licensing serine ADP-ribosylation of target proteins, such as histones (H2BS6ADPr and H3S10ADPr), thereby promoting decompaction of chromatin and the recruitment of repair factors leading to the reparation of DNA strand breaks (PubMed:17177976, PubMed:18172500, PubMed:19344625, PubMed:19661379, PubMed:23230272, PubMed:27067600, PubMed:34465625, PubMed:34874266). HPF1 initiates serine ADP-ribosylation but restricts the polymerase activity of PARP1 in order to limit the length of poly- ADP-ribose chains (PubMed:33683197, PubMed:34732825, PubMed:34795260). In addition to base excision repair (BER) pathway, also involved in double-strand breaks (DSBs) repair: together with TIMELESS, accumulates at DNA damage sites and promotes homologous recombination repair by mediating poly-ADP-ribosylation (PubMed:26344098, PubMed:30356214). Mediates the poly-ADP-ribosylation of a number of proteins, including itself, APLF, CHFR, RPA1 and NFAT5 (PubMed:17396150, PubMed:19764761, PubMed:24906880, PubMed:34049076). In addition to

proteins, also able to ADP-ribosylate DNA: catalyzes ADP-ribosylation of DNA strand break termini containing terminal phosphates and a 2'-OH group in single- and double-stranded DNA, respectively (PubMed:27471034). Required for PARP9 and DTX3L recruitment to DNA damage sites (PubMed:23230272). PARP1- dependent PARP9-DTX3L-mediated ubiquitination promotes the rapid and specific recruitment of 53BP1/TP53BP1, UIMC1/RAP80, and BRCA1 to DNA damage sites (PubMed:23230272). PARP1-mediated DNA repair in neurons plays a role in sleep: senses DNA damage in neurons and promotes sleep, facilitating efficient DNA repair (By similarity). In addition to DNA repair, also involved in other processes, such as transcription regulation, programmed cell death, membrane repair, adipogenesis and innate immunity (PubMed:15607977, PubMed:17177976, PubMed:19344625, PubMed:27256882, PubMed:32315358, PubMed:32844745, PubMed:35124853, PubMed:35393539, PubMed:35460603). Acts as a repressor of transcription: binds to nucleosomes and modulates chromatin structure in a manner similar to histone H1, thereby altering RNA polymerase II (PubMed:15607977, PubMed:22464733). Acts both as a positive and negative regulator of transcription elongation, depending on the context (PubMed:27256882, PubMed:35393539). Acts as a positive regulator of transcription elongation by mediating poly-ADP- ribosylation of NELFE, preventing RNA-binding activity of NELFE and relieving transcription pausing (PubMed:27256882). Acts as a negative regulator of transcription elongation in response to DNA damage by catalyzing poly-ADP-ribosylation of CCNT1, disrupting the phase separation activity of CCNT1 and subsequent activation of CDK9 (PubMed:35393539). Involved in replication fork progression following interaction with CARM1: mediates poly-ADP-ribosylation at replication forks, slowing fork progression (PubMed:33412112). Poly-ADP-ribose chains generated by PARP1 also play a role in poly-ADP-ribose-dependent cell death, a process named parthanatos (By similarity). Also acts as a negative regulator of the cGAS-STING pathway (PubMed:32315358, PubMed:32844745, PubMed:35460603). Acts by mediating poly-ADP- ribosylation of CGAS: PARP1 translocates into the cytosol following phosphorylation by PRKDC and catalyzes poly-ADP-ribosylation and inactivation of CGAS (PubMed:35460603). Acts as a negative regulator of adipogenesis: catalyzes poly-ADP-ribosylation of histone H2B on 'Glu- 35' (H2BE35ADPr) following interaction with NMNAT1, inhibiting phosphorylation of H2B at 'Ser-36' (H2BS36ph), thereby blocking expression of pro-adipogenetic genes (By similarity). Involved in the synthesis of ATP in the nucleus, together with NMNAT1, PARG and NUDT5 (PubMed:27257257). Nuclear ATP generation is required for extensive chromatin remodeling events that are energy-consuming (PubMed:27257257). Plays a role in sister chromatid cohesion by mediating ADP-ribosylation of RSMC during S phase which promotes the interaction between RSMC and CDCA5/sororin, leading to enhanced interaction of CDCA5/sororin with the cohesin complex and promotion of sister chromatid cohesion (PubMed:41261216).

Cellular Location

Chromosome. Nucleus. Nucleus, nucleolus. Cytoplasm, cytosol. Note=Localizes to sites of DNA damage (PubMed:22683995, PubMed:23230272, PubMed:26344098, PubMed:27568560, PubMed:30675909, PubMed:32241924, PubMed:32358582, PubMed:34625544, PubMed:34795260). Recognizes (via PARP-type zinc-fingers) and binds DNA strand breaks (PubMed:22683995). Also binds normal/undamaged chromatin (PubMed:15607977). Auto poly-ADP-ribosylation promotes dissociation from chromatin (PubMed:15607977, PubMed:30675909, PubMed:32358582, PubMed:34625544). Extracted from chromatin by VCP/p97 following sumoylation and ubiquitination (PubMed:35013556). Translocates from the nucleus to the cytosol following phosphorylation by PRKDC (PubMed:35460603). Recruited to replication forks following interaction with CARM1 (PubMed:33412112). [Poly [ADP-ribose] polymerase 1, processed C-

terminus]: Cytoplasm. Note=Following cleavage by caspase-3 (CASP3) and caspase-7 (CASP7) in response to apoptosis, translocates into the cytoplasm, where the auto-poly-ADP- ribosylated form serves as a poly-ADP-ribose carrier to induce AIFM1- mediated apoptosis.

Background

Poly [ADP-ribose] polymerase 1 (PARP1), also known as ADPRT or PPOL is an enzyme that in humans is encoded by the PARP1 gene. PARP1 gene is mapped to 1q42.12. This gene encodes a chromatin-associated enzyme, poly (ADP-ribosyl)transferase, which modifies various nuclear proteins by poly (ADP-ribosyl)ation. The modification is dependent on DNA and is involved in the regulation of various important cellular processes such as differentiation, proliferation, and tumor transformation and also in the regulation of the molecular events involved in the recovery of cell from DNA damage. In addition, this enzyme may be the site of mutation in Fanconi anemia, and may participate in the pathophysiology of type I diabetes.

Images

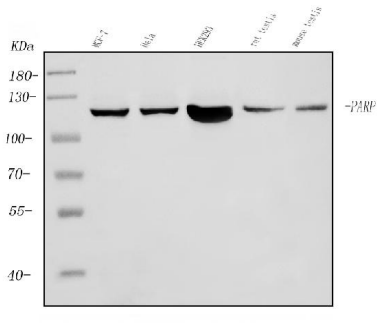


Figure 1. Western blot analysis of PARP using anti-PARP antibody (M00122-4).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human MCF-7 whole cell lysates,
Lane 2: human HELA whole cell lysates,
Lane 3: human HEK293 whole cell lysates,
Lane 4: rat testis tissue lysates,
Lane 5: mouse testis tissue lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-PARP antigen affinity purified monoclonal antibody (Catalog # M00122-4) at 0.25 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for PARP at approximately 116KD. The expected band size for PARP is at 116KD.

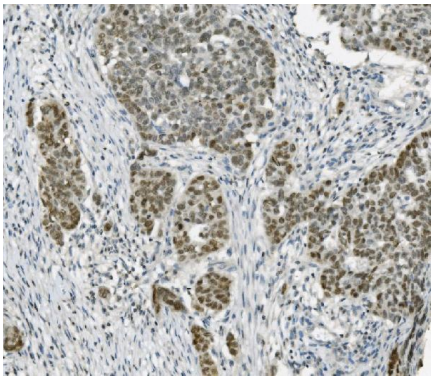


Figure 2. IHC analysis of PARP using anti-PARP antibody (M00122-4).
PARP was detected in paraffin-embedded section of human adnexal serous adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PARP Antibody (M00122-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

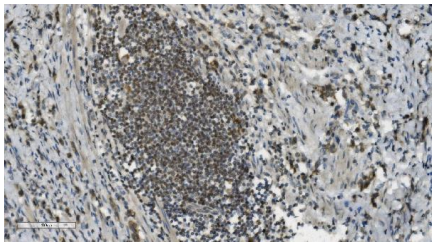


Figure 3. IHC analysis of PARP using anti-PARP antibody (M00122-4).
PARP was detected in paraffin-embedded section of human gastric poorly differentiated adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PARP Antibody (M00122-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

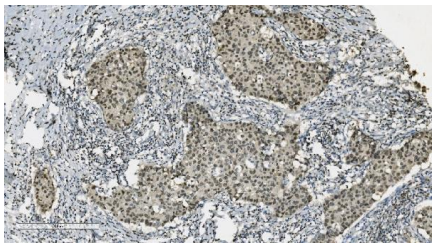


Figure 4. IHC analysis of PARP using anti-PARP antibody (M00122-4).
PARP was detected in paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PARP Antibody (M00122-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

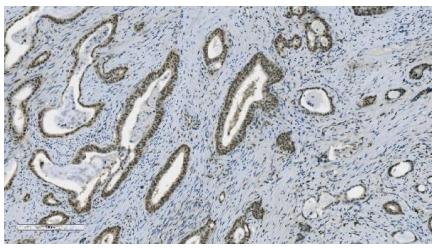


Figure 5. IHC analysis of PARP using anti-PARP antibody (M00122-4).
PARP was detected in paraffin-embedded section of human gallbladder adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PARP Antibody (M00122-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

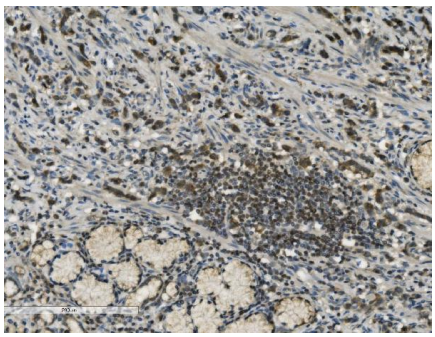


Figure 6. IHC analysis of PARP using anti-PARP antibody (M00122-4).
PARP was detected in paraffin-embedded section of human low-differentiated adenocarcinoma of the gastric tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PARP Antibody (M00122-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

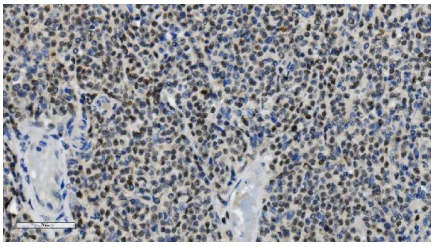


Figure 7. IHC analysis of PARP using anti-PARP antibody (M00122-4).
PARP was detected in paraffin-embedded section of human lymphoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PARP Antibody (M00122-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

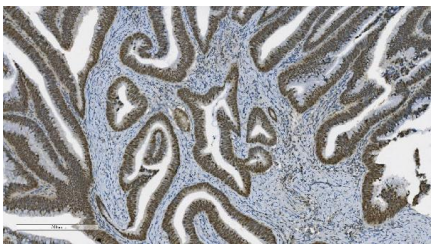


Figure 8. IHC analysis of PARP using anti-PARP antibody (M00122-4).
PARP was detected in paraffin-embedded section of human rectal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-PARP Antibody (M00122-4) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

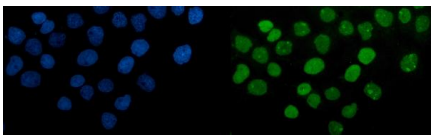


Figure 9. IF analysis of PARP using anti-PARP antibody (M00122-4).
PARP was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-PARP Antibody (M00122-4) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

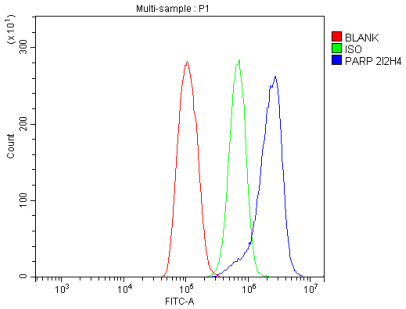


Figure 10. Flow Cytometry analysis of THP-1 cells using anti-PARP antibody (M00122-4).
Overlay histogram showing THP-1 cells stained with M00122-4 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-PARP Antibody (M00122-4, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.