Anti-Poly(ADP-Ribose) Polymer, Clone 10H, Agarose

Mouse Monoclonal Antibody Catalog #1030 LotS0628

LIMITATIONS: THIS PRODUCT IS FOR RESEARCH USE ONLY AND IS NOT APPROVED FOR THERAPEUTIC OR DIAGNOSTIC USE.

Background:

Poly(ADP-ribose) or "pADPr" is a polymer synthesized by a class of enzymes named poly(ADP-ribose) polymerase (PARP). Using NAD+ as substrate, PARP catalyzes the formation of the polymer pADPr, with chain lengths ranging from 2 to 300 residues, containing approximately 2% branching in the chain. pADPr becomes attached to nuclear proteins, and to PARP itself (automodification).

Under normal conditions, cells display low basal level of pADPr polymer, which can dramatically increase in cells exposed to DNA damaging agents (irradiation, alkylation, etc.). This increase of polymer synthesis is usually transient and is followed by a rapid degradation phase with a short half life which can be less than 1 min. The low endogenous level of polymer in unstimulated cells and its rapid catabolism during DNA damage has been ascribed to high activity of the polymer catabolizing enzyme poly(ADP-ribose) glycohydrolase (PARG).

Product Description:

Anti-poly(ADP-ribose) antibody clone 10H (Cat. #1020/N), conjugated to CL-agarose via a 10-atom spacer.

Supplied As:

1 mg of anti-poly(ADP-ribose) clone 10H IgG coupled to 0.375 mL CL-agarose beads. Supplied as a bead slurry in PBS (10 mM phosphate, pH 7.4, 0.138 M NaCl, 2.7 mM KCl, and 0.05% sodium azide) for a total volume of 1 mL.

Storage and Stability:

Stable for 6 months from date of shipment when stored at 4˚C. DO NOT FREEZE!

Specificity and Comments:

Anti-pADPr, clone 10H recognizes poly(ADP-ribose) polymer (pADPr) synthesized by a variety of poly(ADP-ribose) polymerase (PARP)-related enzymes including PARP-1, -2, -3, tankyrase, vPARP, sPARP and others. See Cat. #1020 datasheet for additional antibody details. Use Cat. #1031 Mouse IgG control Agarose as a negative control for immunoprecipitation assays.

Applications:

Useful for immunoprecipitation and immunoaffinity chromatography purification of PARsylated proteins.

Available Control:

Catalog #1031 *Mouse IgG control, Agarose.*
Catalog #2095 *PARP1 Automodified, human, lyophilized.*

Tulip BioLabs Other Related Products:

Catalog #1020 *Anti-poly(ADP-ribose), clone 10H, mouse monoclonal antibody.*
Catalog #1023 *Anti-poly(ADP-ribose), chicken polyclonal antibody.*
Catalog #1051 *Anti-PARP1, chicken polyclonal antibody.*

References:

H. Kawamitsu *et al.* (1984) *Biochemistry* **23,** 3771
M Kanai *et al.* (2003) *Molec. Cell. Biol.* **23**, 2451

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SUGGESTED PROTOCOLS for Anti-pADPr, Clone 10H, Agarose Catalog #1030

NOTE: These methods are intended to be used as a guideline. They have been used successfully in specific experiments, but the exact protocol may need to be altered depending on its intended use.

Buffers:
TBS (20 mM Tris, pH 7.4, 150 mM NaCl)
TBST (TBS + 0.05% Tween 20)

Additional materials (optional):
Cat. #1031 Mouse IgG control, Agarose

IMMUNOPRECIPITATION OF PARslyated PARP1 (CAT. #2095)

1. Reconstitute PARsylated PARP1 (Tulip Cat. #2095) according to instructions.
2. Resuspend 1:1 agarose slurry (Cat. #1030) by gentle agitation, and transfer 20µL or 50µL to a microfuge tube.
3. Wash agarose by adding ~1 mL TBST to the tube, inert a few times, and centrifuge at 10k x g for 45 seconds. Discard supernatant.
4. Add 200 µL TBST to the sedimented beads, then add 10 µL (500ng) of Cat. #2095 Automodified PARP1. Incubate the sample with gentle agitation for at least 2 hrs at RT.
5. Wash beads 3x with TBST as previously. Carefully aspirate all buffer on final wash.
6. Add 75 µL of 2x SDS-PAGE sample buffer to each tube, vortex, and heat tubes for 5 min at ~90˚C.
7. Freeze samples at -20˚C until use.
8. Perform Western blotting using anti-poly(ADP-ribose) antibodies, for example Tulip Cat. #1020 or #1023 according to directions.

APPLICATION NOTES:

1. Immunoprecipitation can be performed using cell extracts prepared in cell lysis buffer.
2. Western blotting can be performed using desired antibodies.
3. Elution of immunoprecipitated cellular proteins from the agarose beads (Cat. #1030) has been reported using pH 3 elution buffer (Kanai *et al*, 2003).
4. Use Cat. #1031 Mouse IgG control, Agarose in parallel samples to determine non-specific binding to the #1030 beads.

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