

**Anti-Poly(ADP-Ribose) Polymer,
Clone 10H [carrier-protein free]****Mouse Monoclonal Antibody
Catalog #1020/N LotT1019**

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).

Clone Designation:

10H.

Ig Isotype/Light Chain:

IgG3_κ

Hybridoma:

Balb/c x NS-1.

Immunogen:

Poly(ADP-ribose) mixed with methylated bovine serum albumin.

Supplied As:

1 mg/ml of IgG purified from hybridoma cell culture supernatant by protein A chromatography. Prepared in 10 mM phosphate, pH 7.4, 0.138 M NaCl, 2.7 mM KCl, and 0.02% sodium azide. This is a carrier-protein free formulation (neat).

Storage and Stability:

Stable for 1 year from date of shipment when stored at -20 or -70°C. Stable for 1 month at 4°C. Avoid freeze/thaw cycles.

Specificity and Comments:

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. The 10H has been used for WB, ELISA-based PARP activity screen, immunodot blot, immunofluorescence.

Crossreactivity:

Does not crossreact with ADP-ribose, 5'-AMP, or yeast RNA as tested by ELISA.

Applications and Suggested Dilutions:

Immunocytochemistry 5-20 µg/ml
Western Blot (10 µg/ml using colorimetric methods; <2 µg/ml for ECL).
Immunoprecipitation, ELISA.

Please note: This information is intended as a guide. The optimal dilutions must be determined by the user.

Available Control:

Catalog #2095 PARP1 Automodified, human, lyophilized.

Tulip BioLabs Other Related Products:

Catalog #1023 Anti-poly(ADP-ribose), chicken polyclonal antibody.

Catalog #1051 Anti-PARP1, chicken polyclonal antibody.

Original Reference:

H. Kawamitsu *et al.* (1984) *Biochemistry* **23**, 3771

Tulip BioLabs References:

A.H. Forst *et al.* (2013) *Structure* **21**, 462
L. Kashima *et al.* (2012) *J. Biol. Chem.* **287**, 12975
E. Kotova *et al.* (2009) *PLoS Genetics* **5** e1000387
J.-P. Gagne *et al.* (2008) *Nucleic Acids Res.* **36**, 6959
P. Chang *et al.* (2005) *Nature Cell Biol.* **7**, 1133
C. Zhang *et al.* (2004) *Am. J. Physiol. Heart Circ. Physiol.* **287**, H659
J.-P. Gagne *et al.* (2012) *Nucleic Acids Res.* doi: **10.1093/nar/gks486**
L. Kashima *et al.* (2012) *J. Biol. Chem.* **287**, 12975

Other General References:

G.M. Shah *et al.* (1995) *Anal. Biochem.* **227**, 1
D. D'Amours *et al.* (1999) *Biochem. J.* **342**, 249
L. Virag and C. Szabo (2002) *Pharmacol. Rev.* **54**, 375

SUGGESTED PROTOCOLS for Anti-pADPr, Clone 10H, Catalog #1020

NOTE: These methods are meant 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.

WESTERN BLOTTING

Buffers:

TBS (20 mM Tris, pH 7.4, 150 mM NaCl)

TBST (TBS + 0.05% Tween 20)

Blotto (TBST + 1% Carnation nonfat milk powder)

Blotto/BSA (Blotto + 1% BSA)

1. Transfer protein from 4-15% SDS-PAGE to nitrocellulose sheet (NC). Note: Poly-ADP-ribosylation of target proteins show an increase in apparent MW in SDS-PAGE.
2. Block NC blot by incubating with blotto for 1-2 h @ RT.
3. Add primary antibody (#1020) to 10 µg/ml (diluted with blotto/BSA), and incubate blot with gentle agitation for 1 h @ RT. Note: The inclusion of BSA in the primary antibody dilution buffer eliminates crossreaction with BSA which may be present in the experimental sample.
4. Wash blot 4-times with TBST with 2 min agitation between buffer changes.
5. Add secondary antibody (GAM-HRP; Jackson Immunoresearch) diluted 1/1000 (in blotto), and incubate 30 min @ RT with gentle agitation.
6. Wash blot 4-times with TBST, then 1-time with TBS.
7. Add TMB reagent (BioFX TMBM) and develop until desired staining is obtained.
8. Rinse with H₂O, then air dry.
9. Photograph or scan image for permanent record.

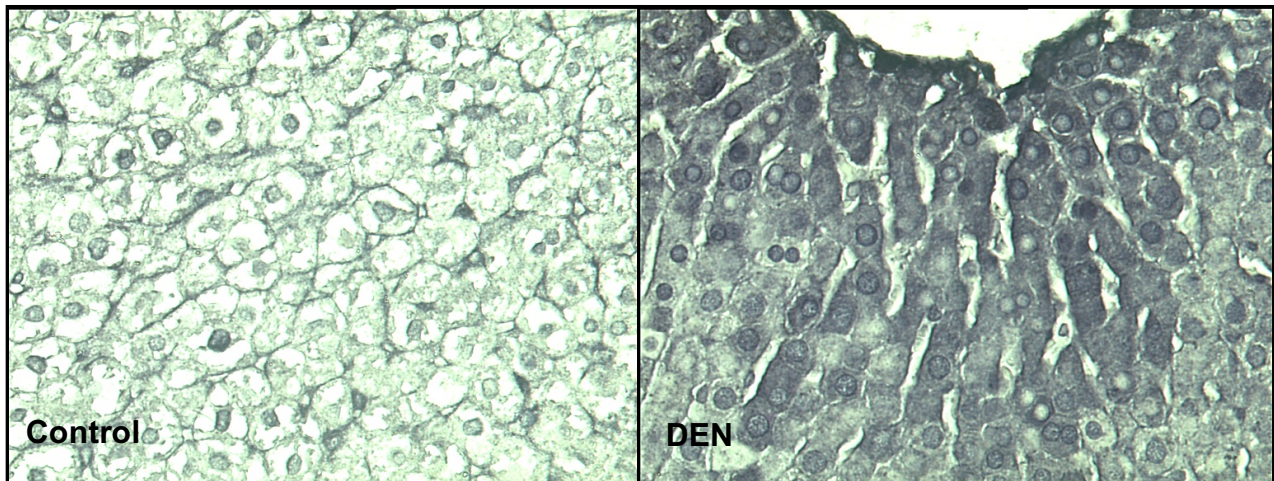
SUGGESTED PROTOCOLS for Anti-pADPr, Clone 10H, Catalog #1020

NOTE: These methods are meant 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.

IMMUNOHISTOCHEMISTRY (Detection of poly(ADP-ribose) in liver)

(see Rawling et al. (1993) Carcinogenesis 14 2513)

1. Fix thin pieces of fresh liver tissue in 10% buffered formalin overnight.
2. Process sections:
 - xylene wash 3 x 5 min
 - EtOH series: 100% x 2 min, 95% x 2 min, 75% x 2 min
3. Wash in PBS for 10 min.
4. Treat with 1% H₂O₂ for 20 min
5. Block for 1 h with horse serum (Vector Elite ABC kit).
6. Blot excess blocking solution from slide to prevent dilution of primary antibody.
7. Add primary antibody (#1020) diluted to 100 µg/ml (1/100) in blocking buffer for 2 h at RT.
8. Wash in PBS 5 x 5 min.
9. Add secondary biotinylated antibody (Vector) for 30 min at RT.
10. Wash in PBS for 5 min.
11. Add Elite ABC reagent (avidin) for 30 min at RT.
12. Add peroxidase substrate (Ni-DAB) for 11 min at RT.
13. Rinse with water 5 min.
14. Reverse series of EtOH washes (75%-95%-100%) and clear in xylene.
15. Apply permount and cover slip.



Poly(ADP-ribose) staining of rat liver. Rats were injected i.p. with diethylnitrosamine (200 mg/kg), and the livers were removed and rapidly processed 10 hr later, at peak polymer induction (DEN). Control was untreated liver. Tissue were stained according to the above protocol using anti-pADPr clone 10H. (courtesy J.B. Kirkland, Univ. Guelph, Canada)