# Anti-Poly(ADP-Ribose) Polymer, Clone 10H, Biotin Conjugate

# Mouse Monoclonal Antibody Catalog #1021 LotQ0211

*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:***
IgG3κ

***Hybridoma:***
Balb/c x NS-1.

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

***Supplied As***:
0.5 mg/ml of IgG conjugated to biotin. Prepared in 10 mM phosphate, pH 7.4, .15 M NaCl, 1% BSA and 0.02% sodium azide..

***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 1-5 µg/ml
Western Blot (2 µg/ml using colorimetric methods; <1 µg/ml for ECL), 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.*

***Tulip BioLabs Other Related Products***:
**Catalog #1020** *Anti-poly(ADP-ribose), clone 10H*
**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 Refs for unconjugated IgG:***
A.H. Forst *et al.* (2013) Structure **21**, 462
L. Kashima *et al.* (2012) *J. Biol. Chem.* **287**, 12975
P. Chang *et al.* (2005) *Nature Cell Biol.* **7**, 1133
J.-P. Gagne *et al.* (2012) *Nucleic Acids Res.* **doi: 10.1093/nar/gks486**
L Kashima *et al.* (2012) *J. Biol. Chem.* **287**, 12975

**Tulip BioLabs, Inc.**
**P.O. Box 334, West Point, PA 19486 USA**
**Tel/Fax 610.584.2706 info@tulipbiolabs.com**
**www.tulipbiolabs.com**

# SUGGESTED PROTOCOLS for Anti-pADPr, Clone 10H, Catalog #1021

*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.*

*Buffers*:
PBS (10 mM sodium phosphate, pH 7.4, 150 mM NaCl)
PBST (PBS + 0.05% Tween 20)
Blocking buffer (PBST + 1% bovine serum albumin)

## WESTERN BLOTTING

1. Transfer protein from 4-15% SDS-PAGE to nitrocellulose sheet (NC). Note: Poly-ADP-ribosylation of target proteins display an increase in apparent MW in SDS-PAGE.
2. Block NC blot by incubating with blocking buffer for 1-2 h @ RT.
3. Add primary antibody (#1021) to 1-5 µg/ml (diluted with blocking buffer), and incubate blot with gentle agitation for 1 h @ RT.
4. Wash blot 4-times with PBST with 2 min agitation between buffer changes.
5. Add biotin x HRP conjugate (Jackson Immunoresearch) diluted 1/2000 in blocking buffer (or as directed by datasheet), and incubate 30 min @ RT with gentle agitation.
6. Wash blot 4-times with PBST, then 1-time with PBS.
7. Add membraneTMB reagent (BioFX TMBM) and develop until desired staining is obtained.
8. Rinse with $H_2O$, then air dry.
9. Photograph or scan image for permanent record.

## ELISA

1. Coat microwells of a 96-well high binding plate with automodified PARP1 (Tulip Cat. #2095) as follows:
   Reconstitute autmodified PARP1 (#2095) according to instructions.
   Dilute the automodified PARP to 20ng/mL in PBS
   Add 50µL/well automodified PARP to the microplate, let set O/N at 4˚C.
   Wash plate 2x with 325µL PBS.
   Block plate with PBST/1%BSA 100µL/well for 1h at RT.
   Shake out buffer and use immediately, or air dry and store desiccated.

2. Make serial dilutions of #1021 biotin conjugate in PBST/1% BSA starting at 1/1000 (optimal is ~1/20000).
3. Add 100µL/well of the diluted conjugate, let set with gentle agitation for 1h at RT.
4. Wash the plate with 325µL PBST/well x 4.
5. Add 100µL/well HRP-streptavidin at 1/2000 dilution in PBST/1%BSA, let set with gentle agitation for 30 min.
6. Wash the plate with 325µL PBST/well x 4, then PBS x 1.
7. Add 100µL TMB colorimetric reagent/well, let incubate to desired intensity (<30 min).
8. Stop rxn with 100µL 0.2N HCl, the wells will turn yellow in color.
9. Read OD450 in a microplate reader.