ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Poly ADP-ribose Antibodies

Cat. # ADPR11-C	Poly ADP-ribosylated proteins for Western	**SIZE**: 100 ul
Cat. # ADPR12-M	Mouse Monoclonal Anti-Poly ADP-ribose IgG #2	**SIZE**: 100 ug

The 3'ends of chromosomes are capped with **telomere** sequences (TTAGGG; 6-26 nucleotides in length) by ribonucleoprotein telomerase during DNA replication. **Telomerase** is an unusual RNA-dependent DNA polymerase that uses and RNA component to specify the addition of telomere. The telomeric RNA contains a sequence complementary to TTAGGG. Introduction of telomerase into normal human cells has been shown to extend normal cell life by ~ 20 doubling.

Poly(ADP-ribose) polymerases (**PARPs**) catalyze formation of long, branched chain of poly(ADP-ribose) onto protein acceptors using NAD+ as a substrate. Poly(ADP)ribosylation is a transient posttranslational modification that can either enhance or reduce protein activity. **Tankyrase** (TRF1 interacting ankyrin-related ADP-ribose polymerase; human 1327 aa, **renamed as TNKS-1/TANK1,** chromosome 8), a modular protein with homology to ankyrin and poly(adenosine diphosphate-ribose) polymerase (PARP) has been cloned and localized to telomere. TANK1 is alternatively spliced to isoform 1 and 2 (missing 644-1327). The N-terminal **HPS domain** contains multiple run of histidine, proline, and serine residue homopolymers. TANK1 has 24 ankyrin repeats in TRF-1 interacting domain near the N-terminus. The 33-aa ANK repeat motif mediates protein-protein interactions. The ANK domain is followed another protein interaction motif called the sterile alpha-module (**SAM**). The C-terminal region of TANK1 contains the PARP activity. TANK1 uses its ANK domain to bind TRF1 and its PARP domain to ADP-ribosylate itself and TRF1, and thereby inhibiting the ability of TRF1 to bind telomere. The homology between tankyrase and PARPs is limited to catalytic domain. Takyrase-1 is expressed in many tissues and targeted to various intracellular compartments. Tanyrase-1, devoid of NLS (nuclear localization signal), is translocated to telomere (nucleus) through binding of its ANK domain to TRF1.

Source of Antigen, Antibodies, and Positive Controls

Antigen	Purified bovine Purified poly (ADP-ribose) polymer was injected into **mice**
Ab Host/type	A clone secreting IgG3,k to poly-ADP ribose was identified, selected, and expanded as ascites. Antibody (cat # ADPR12-M) was purified by protein A/G column.
2-Ab	**Goat Anti-mouse IgG-HRP conjugate** Cat # 40320 (AP, biotin, FITC conjugates also available)
-ve	Cat # 20008-1, Mouse (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control

For western blot +ve control (**cat # ADPR11-C**),. PARP was expressed in E. coli and ribosylated proteins were isolated and supplied SDS-PAGE sample buffer (reduced). The protein extract contains many proteins of varying mol weight that are detected by the antibodies. Load 10 ul/lane of ADPR11-C for good visibility with antibody Cat # ADPR11-S. Store at –20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the ADPR11-C solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly.

Form & Storage of Antibodies/Peptide Control

IgG (purified)
100 ug/vial solution lyophilized powder
PBS, 1o mM Heps 0.05% sodium azide and 1% BSA
Reconstitute in water at 1 mg/ml.

Storage
Short-term: unopened, undiluted vials for less than a week at 4oC.
Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder.

Recommended Usage

Western Blotting (1:1K-5K for antiserum using ECL technique).

ELISA: Control proteins can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

Histochemistry & Immunofluorescence: Not tested. We recommend the use of affinity purified ab at 2-20 ug/ml.

Specificity & Cross-reactivity

The ADPR12-M antibodies will detect proteins modified with ADP-ribose with a minimum of ~10 units in all species. The E. coli proteins modified with poly ADP-ribose (**cat # ADPR11-C**) can be used as +ve control for this antibody.

General References: (1) Affar EB et al (1998) Anal. Biochem. 259, 280; Shah GM et al (1995) Biochem J. Anal. Biochem. 232, 251; Smith S et al (1998) Science 282, 1484; Smith S et al (1999) J. Cell Sci. 112, 3649; Chi NW et al (2000) JBC 275, 38437; Cook BD et al (2002) Mol Cell. Biol. 22, 332-342

*This product is for In vitro research use only.

Related material available from ADI

Antibodies TANK1/2, TRF1-2, TP1, Est2, GRBP14, Tab182, Glut4

ADPR12-M-C 70831A

Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com