For life science research only.
Not for use in diagnostic procedures.

Anti-Poly-(ADP-Ribose) Polymerase
from rabbit

Version: 06
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Polyclonal antibody against poly-(ADP-ribose) polymerase for western blotting, immunofluorescence, and immunoprecipitation

Cat. No. 11 835 238 001	100 µl 50 blots

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	colorless	Anti-Poly-(ADP-Ribose) Polymerase	Stabilized serum.	1 vial, 100 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	colorless	Anti-Poly-(ADP-Ribose) Polymerase	Store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For western blotting

For additional information on preparing solutions, see section, **Working Solution**.

- Extraction buffer
- Blocking buffer
- Tris-HCl*
- Tween 20*
- PMSF*
- SDS*
- Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit)* or
- BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)*
- SDS/polyacrylamide minigels (10%), 7 cm × 7 cm
- Nitrocellulose sheets, 8 cm × 8 cm
- Miniblot apparatus

For staining procedures for immunofluorescence

- Phosphate-buffered saline (PBS*)
- Ice-cold pure methanol or 4% paraformaldehyde (v/v)/PBS
- PBT (PBS, 0.1% Bovine Serum Albumin*, 1% Tween 20*)
- Anti-rabbit-Ig-fluorescein
- Fluorescence microscope

1.4. Application

The antibody is suitable for immunohistocytochemistry, immunoprecipitation, and western blotting.

2. How to Use this Product

2.1. Before you Begin

Working Solution

Solution	Content	Preparation of Working Solution
1	Blocking buffer	50 mM Tris-HCl, pH 8, 150 mM NaCl, 0.3% Tween 20 (v/v), and 5% dry milk.
2	Immunoreagent	For western blots on nitrocellulose sheets of 8 cm × 8 cm, use 10 µl of serum diluted in 20 ml of Blocking buffer. *Solution can be used up to 5 times when stored at −15 to −25°C.*
3	Extraction buffer	For each sample, mix: ▪ 100 µl of 50 mM glucose, 25 mM Tris-HCl, pH 8, 10 mM EDTA, 1 mM PMSF with ▪ 50 µl of 50 mM Tris-HCl, pH 6.8, 6 M urea, 6% 2-mercaptoethanol, 3% SDS, 0.003% bromophenol blue.

2.2. Protocols

Western blotting

Preparation of crude extracts

1. Obtain crude cell extracts by suspending approximately 1 × 10^7 cells in 150 µl of Extraction buffer.
2. Sonicate for one 60 second pulse at 180 V.
3. Incubate for 15 minutes at +65°C before loading.

SDS gel electrophoresis and electronic transfer

1. Use 7 × 7 cm SDS/polyacrylamide minigels (10%) according to the protocol of Laemmli (Laemmli UK, 1979).
2. Perform electrotransfer of proteins onto nitrocellulose sheets according to Towbin (Towbin H, 1979), at +2 to +8°C in a miniblot apparatus for 1 hour at 200 mA.

Immunoreaction

1. Block blots by incubation with Blocking buffer for 1 hour at +15 to +25°C.
2. For immunoreaction, incubate with the Immunoreagent solution for 2 hours at +15 to +25°C.
3. Wash twice in 20 ml of Blocking buffer.
4. Proceed with the BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit), or Lumi-Light^{PLUS} Western Blotting Kit (Mouse/Rabbit).

Staining procedures for immunofluorescence

1. After washing cells with PBS, fix cells in ice-cold pure methanol at −15 to −25°C for 30 minutes or in 4% paraformaldehyde (v/v)/PBS for 15 minutes at +15 to +25°C.

2. Wash cells twice with PBT (PBS, 0.1% bovine serum albumin, 1% Tween 20).
   – Incubate with the Immunoreagent solution for 60 minutes at +15 to +25°C.

3. Wash cells twice with PBT.
   – Incubate with 10 µg/ml anti-rabbit-Ig-fluorescein for 30 minutes at +15 to +25°C.

4. Wash cells twice with PBS and examine on a slide using a fluorescence microscope.

⚠ ***The antibody solutions should be free of precipitates. If necessary, centrifuge the solutions at high speed prior to use. Do not allow the section to dry out during this procedure.***

2.3. Parameters

Specificity

In immunoprecipitation and western blot experiments, the antibody recognizes full length PARP (primates and rodents), as well as the large PARP fragment generated by caspases.

3. Additional Information on this Product

3.1. Test Principle

Poly-(ADP-Ribose) Polymerase (PARP) is a zinc-dependent, eukaryotic, DNA-binding protein that specifically recognizes DNA strand breaks produced by various genotoxic agents. It has been reported that PARP serves as a substrate for apoptosis-specific proteases from the ICE family. The protease responsible for the cleavage of PARP was termed apopain, which is derived from its proenzyme Yama/CPP32β. Additionally, CPP32/Mch2 as well as TX and Nedd-2 were described as PARP-cleaving ICE-like proteases. Publishers found that the 113 kD PARP is cleaved during apoptosis into 89 kDa and 24 kDa fragments, which could serve as an early specific marker of apoptosis. Therefore, anti-PARP was used in western-blot analysis. However, PARP cleavage cannot be the only trigger for apoptosis since PARP-deficient knockout mice develop normally.

Preparation

Polyclonal serum (VIC 5) is produced by immunization of rabbits with a full length recombinant PARP produced in the Sf9/Baculovirus system.

3.2. References

- Laemmli UK. Most commonly used discontinuous buffer system for SDS electrophoresis. Nature. 1979;227:680-686.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA. 1979;9:4350-4354.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Sodium Dodecyl Sulfate (SDS)	1 kg	11 667 289 001
Tris hydrochloride	500 g	10 812 846 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
PMSF	10 g	10 837 091 001
	25 g	11 359 061 001
BM Chemiluminescence Western Blotting Kit (Mouse/Rabbit)	1 kit, 2,000 cm^2 membrane (trays), 12,500 cm^2 membrane (transparent plastic bags)	11 520 709 001
Bovine Serum Albumin Fraction V	50 g	10 735 078 001
	100 g, *Not available in US*	10 735 086 001
	500 g, *Not available in US*	10 735 094 001
	1 kg, *Not available in US*	10 735 108 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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