

# PARP1-Trap Agarose

Product code: xta

## Introduction

The ChromoTek PARP1-Trap Agarose consists of an anti-PARP1 Nanobody (VHH), which is covalently bound to agarose beads. PARP1-Trap Agarose is used to immunoprecipitate PARP1 from cell extracts.

## Properties

**Ligand:** Anti-PARP1 single domain antibody fragment (VHH, Nanobody)

**Reactivity:** Specifically binds to Poly(ADP-ribose) polymerase 1 (PARP1) from human. *Note: PARP1-Trap does not bind to other members of the PARP family, e.g. PARP2, PARP3, or PARP9.*

**Epitope:** Within the DNA binding domain of PARP1

**Bead size:** 90  $\mu\text{m}$  (cross-linked 4 % agarose beads)

**Storage buffer:** 20 % ethanol

**Storage conditions:** Upon receipt store at +4°C. Do not freeze!

**Stability:** Stable for 1 year upon receipt.

**Shipment:** Shipped at ambient temperature.

**RRID:** AB\_2631384

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## Suggested buffer compositions

### Required buffer solutions

*NEW: Update of Wash buffer components.*

Buffer	Composition
Lysis buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % Nonidet™ P40 Substitute (adjust the pH at +4°C)
RIPA buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1 % SDS, 1 % Triton™ X-100, 1 % deoxycholate (adjust the pH at +4°C)
Dilution buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA (adjust the pH at +4°C)
Wash buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.05 % Nonidet™ P40 Substitute, 0.5 mM EDTA (adjust the pH at +4°C)
2x SDS-sample buffer	120 mM Tris/Cl pH 6.8, 20 % glycerol, 4 % SDS, 0.04 % bromophenol blue, 10 % β-mercaptoethanol
Acidic elution buffer	200 mM glycine pH 2.5 (adjust the pH at +4°C)
Neutralization buffer	1 M Tris pH 10.4 (adjust the pH at +4°C)
Urea elution buffer	8 M urea

*Note: Use your equivalent cell lysis buffer for other cell types like yeast, plants, insects, bacteria.*

*Note: Consider using a Wash buffer without detergent for co-immunoprecipitation.*

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## Product sizes

Product	Product code	Size
PARP1-Trap Agarose	xta2-10	10 reactions (250 µL slurry)
	xta2-20	20 reactions (500 µL slurry)
	xta2-100	100 reactions (2.5 mL slurry)
	xta2-200	200 reactions (5 mL slurry)
	xta2-400	400 reactions (10 mL slurry)
PARP1-Trap Agarose Kit	xtak-20	20 reactions (500 µL slurry) including buffers

# PARP1-Trap Agarose

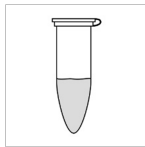
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## Protocol at a glance

### General

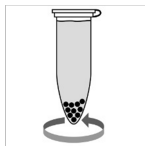
- Perform all steps at +4°.
- Use your preferred cell lysis buffer and cell lysis conditions.

### Cell Lysis



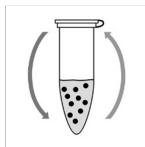
- Use  $10^6$ - $10^7$  cells and 200  $\mu$ L Lysis buffer.
- Perform cell lysis and clear lysate.
- Mix 200  $\mu$ L cleared lysate with 300  $\mu$ L Dilution buffer.

### Bead equilibration



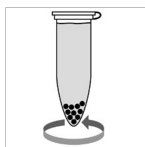
- Transfer 25  $\mu$ L bead slurry into a 1.5 mL tube.
- Equilibrate beads 3x with 500  $\mu$ L Dilution Buffer.

### Protein binding



- Add 500  $\mu$ L diluted lysate to beads.
- Rotate end-over-end for 1 hour at +4°C.

### Washing



- Wash beads 3x with 500  $\mu$ L Wash buffer.
- Transfer beads to a new tube during the last washing step.

### Elution with SDS-sample buffer



- Resuspend beads in 80  $\mu$ L 2x SDS-sample buffer.
- Boil beads for 5 min at +95°C.
- Analyze the supernatant in SDS-PAGE / Western Blot.

## Immunoprecipitation protocol

### Cell material

The following protocol describes the preparation of mammalian cell lysate!

For other type of cells, we recommend using 500 µg of cell extract and start the protocol with step *Bead equilibration*.

### Mammalian cell lysis

Note: Harvesting of cells and cell lysis should be performed with ice-cold buffers. We strongly recommend to add protease inhibitors to the Lysis buffer to prevent degradation of your target protein and its binding partners.

For one immunoprecipitation reaction, we recommend using  $\sim 10^6$ - $10^7$  cells.

#### 1. Choice of lysis buffer:

- For cytoplasmic proteins, resuspend the cell pellet in 200 µL ice-cold Lysis buffer by pipetting up and down. Supplement Lysis buffer with protease inhibitor cocktail and 1 mM PMSF (not included).
- For nuclear/chromatin proteins, resuspend cell pellet in 200 µL ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 Kunitz U/mL), MgCl<sub>2</sub> (f.c. 2.5 mM), protease inhibitor cocktail and PMSF (f.c. 1 mM) (not included).

2. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.

3. Centrifuge cell lysate at 17,000x g for 10 min at +4°C. Transfer cleared lysate (supernatant) to a pre-cooled tube and add 300 µL Dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included). If required, save 50 µL of diluted lysate for further analysis (input fraction).

### Bead equilibration

1. Resuspend the beads by gently pipetting up and down or by inverting the tube. Do not vortex the beads!

2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube.

3. Add 500 µL ice-cold Dilution buffer.

4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard the supernatant.

*Note:* Alternatively, Spin columns (sct-10; -20; -50) can be used to equilibrate the beads.

### Protein binding

1. Add diluted lysate to the equilibrated beads.

2. Rotate end-over-end for 1 hour at +4°C.

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## Washing

1. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
2. If required, save 50 µL of supernatant for further analysis (flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 µL Wash buffer.
5. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C. Discard remaining supernatant.
6. Repeat this step at least twice.
7. During the last washing step, transfer the beads to a new tube.

*Note:* Alternatively, Spin columns (sct-10; -20; -50) can be used to wash the beads.

## Elution with 2x SDS-sample buffer (Laemmli)

1. Remove the remaining supernatant.
2. Resuspend beads in 80 µL 2x SDS-sample buffer.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
5. Analyze the supernatant in SDS-PAGE / Western Blot.

## Elution with Acidic elution buffer

1. Remove the remaining supernatant.
2. Add 50-100 µL Acidic elution buffer and constantly pipette up and down for 30-60 sec at +4°C or room temperature.
3. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate fraction with 5-10 µL Neutralization buffer.
6. Repeat this step at least once to increase elution efficiency.

*Note:* Elution at room temperature is more efficient than elution at +4°C. Prewarm buffers for elution at room temperature.

*Note:* Alternatively, Spin columns (sct-10; -20; -50) can be used to separate the beads.

## Elution with Urea elution buffer

1. Remove the remaining supernatant.
2. Add 50-100 µL Urea elution buffer and mix with a pipette.
3. Mix for 5 min at room temperature
4. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
5. Transfer the supernatant to a new tube.
6. Repeat this step at least once to increase elution efficiency.

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*Note:* Elution at room temperature is more efficient than elution at +4°C. Prewarm buffers for elution at room temperature.

*Note:* Alternatively, Spin columns (sct-10; -20; -50) can be used to separate the beads.

## Product overview and related products

PARP1 toolbox	Product code
PARP1-Trap Agarose	xta-10; -20; -100
PARP1-Trap Agarose Kit	xtak-20
Binding Control Agarose	bab-20
Spin columns	sct-10; sct-20; sct-50
PARP1 VHH, recombinant binding protein	xt-250
PARP1-Chromobody® plasmid (TagGFP2)	xcg
PARP1-Chromobody® plasmid (TagRFP)	xcr

For product details, information, and ordering visit [www.chromotek.com](http://www.chromotek.com).

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