PARP1-Trap_A Kit for Immunoprecipitation of PARP1-Fusion Proteins from mammalian cell extract

Only for research applications, not for diagnostic or therapeutic use.

Introduction

Poly(ADP-ribose) polymerase 1 (PARP1) is one of the most abundant proteins in the nucleus and is involved in many cellular processes like DNA repair, transcriptional regulation, and modulation of chromatin structure. PARP1-Trap is excellent for fast and efficient one-step isolation of PARP1 and its interacting factors from cellular extract. Isolated PARP1 protein may be used further for immunoblot analysis, mass spectrometry, and enzyme assays. PARP1-Trap utilizes small recombinant antibody fragments covalently coupled to the surface of agarose beads.

Specificity

PARP1 Trap binds to PARP1 and not to other members of the PARP family.

Content

Reagent	Code	Quantity
PARP1-Trap_A kit	xtak-20	20 reactions (0.5 ml slurry)
Lysis buffer (CoIP) For lysis of mammalian cells		30 ml
RIPA buffer (ChIP) For lysis of mammalian cells		30 ml
5x Wash / Dilution buffer		2 x 10 ml
Elution buffer		3 ml

Beads Properties

Bead size: 90 µm (cross-linked 4% agarose beads)
Storage buffer: 20% EtOH

Stability and Storage

Shipped at ambient temperature. Upon receipt store at +4°C.
Stable for 1 year. Do not freeze.

Required solutions/ Buffer

Suggested buffer composition (as provided in the kit)

Buffer	Composition
Lysis buffer (CoIP) For lysis of mammalian cells	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0,5 mM EDTA; 0.5% NP-40; 0,02% Thimerosal
RIPA buffer For lysis of mammalian cells	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0,5 mM EDTA; 0,1% SDS; 1% Triton X-100; 1% Deoxycholate; 0,02% Thimerosal
1x Wash/ Dilution buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0,02% Thimerosal
Elution Buffer	200 mM Glycine pH 2.5

Note: Add 40 ml H_2O to 5x Wash/ Dilution buffer before use. It is 5 times concentrated!!
Note: 0,02% Thimerosal is added to our buffers as an antiseptic and antifungal agent.
Note: For other cell types like yeast, plants, drosophila, etc. please use your equivalent cell lysis buffer.

Suggested buffer composition (not provided in the kit)

Buffer	Composition
Neutralization Buffer	1M Tris pH10
2 x SDS-sample buffer (Laemli)	120 mM Tris/Cl pH 6.8; 20% glycerol; 4% SDS, 0.04% bromophenol blue; 10% β-mercaptoethanol
Elution Buffer (alternative)	8 M Urea

Related products

PARP1 Toolbox	Code
PARP1 bimding protein	xt-250
PARP1-Trap_A	xta-20; xta-100; xta200
Binding control: agarose beads	bab-20
Spin columns	sct-10; sct-20; sct-50

Support

Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com*

We are also happy to hear your feedback and to include your protocol for the cell lysis in our manual.

Protocol for immunoprecipitation and elution of proteins from PARP1-Trap_A

Steps 1-5 describe the preparation of mammalian cell lysate.
For other types of cells we recommend using 0.5 – 1 mg of protein extract and start the protocol with Step 6.

Harvest mammalian cells	*Note: If you want to make an IP from other cell types like yeast, plants, etc. please use your own protocol for cell lysis and equivalent lysis buffer.* **1.** For one immunoprecipitation reaction the use of ~10^6 - 10^7 cells (approx. one 10-cm dish) expressing the protein of interest is recommended. **2.** To harvest adherent cells, aspirate growth medium, add 1 ml ice-cold PBS to cells and scrape cells from dish. Transfer cells to a pre-cooled tube, spin at 500 g for 3 min at +4°C and discard supernatant. Wash cell pellet twice with ice-cold PBS: Add 500 µl ice-cold PBS, gently resuspend the cells, centrifuge at 500 g for 3 min at 4°C. Carefully remove supernatant and discard. Repeat wash step twice. *Note: The cell pellet can be stored long term at -80°C.*
Lyse cells	**3. Resuspend the washed cell pellet in 200 µl ice-cold lysis buffer by pipetting.** Supplement lysis buffer with protease inhibitors and DNase (not included). *Optional for nuclear/chromatin proteins: Use RIPA buffer supplemented with 1 mg/ml DNase, 2.5 mM $MgCl_2$ and protease inhibitors (not included).* **4. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.** **Centrifuge lysated cells at 20.000x g for 10 min at +4°C. Transfer supernatant (lysate) to a pre-cooled tube and add 300 µl 1x wash/dilution buffer to supernatant (lysate). Discard pellet with cell debris.** If required, save 50 µl of the diluted lysate for immunoblot analysis (Input). *Note: Add 40 ml H_2O to 5x wash/dilution buffer before first use. It is 5 times concentrated!!* *Optional: Add protease inhibitors (not included) to 1x wash/ dilution buffer.* *Note: At this point cell lysate can be stored long term at -80°C.*
Equilibrate beads	**5.** Beads can be equilibrated during incubation step of lysis procedure. **Vortex PARP1-Trap_A beads intensively and directly pipette 25 µl bead slurry into a new tube with 500 µl ice-cold 1x wash/dilution buffer and pipette up and down a few times.** *Note: Add 40 ml H_2O to 5x wash/dilution buffer before first use. It is 5 times concentrated!!* *Note: The slurry is more efficiently drawn into a wide bore pipette tip. We suggest clipping a little off the end of a regular tip to mimic the benefit of a wide bore tip. It is important to thoroughly resuspend the Nano-Trap-beads slurry by vortexing.* **6. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash step twice.** Carefully remove the supernatant with a hollow needle G27 or a small pipetting tip so that the beads pellet is not sucked up.
Bind proteins	**7. Add diluted supernatant (from step 4) to equilibrated PARP1-Trap_A beads (from step 6). Tumble end-over-end for at least 30 min at 4°C**. **8. Centrifuge at 2.500x g for 2 min at +4°C.** If required, save 50 µl supernatant for immunoblot analysis (Flow Through). **Carefully remove the supernatant** with a hollow needle G27 or a small pipetting tip so that the beads pellet is not sucked up. **Discard remaining supernatant** with unbound fractions. The beads with the bound proteins are in the pellet.

Wash beads	**9. Resuspend PARP1-Trap_A beads in 500 µl ice-cold 1x wash/dilution buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash step twice. Within the third washing step, transfer the diluted beads to a new tube.** *Optional: Increase salt concentration in the second washing step up to 500 mM.*
Elute proteins	**Depending on your downstream application different elution methods are possible:** **(1)** Elution with Glycine- Elution Buffer: **Resuspend the PARP-Trap_A beads with 50–100 µl Elution Buffer** (200 mM Glycine, pH 2.5) **by pipetting up and down for 30 sec at room temperature.** Make sure that all of the PARP1-Trap_A beads are resuspended. **Transfer the supernatant to a new tube. Then, immediately, neutralize the solution with 5-10 µl 1M Tris pH 10.4.** To increase elution efficiency this step can be repeated. *Note: It is important that the elution step and the neutralization is done at room temperature and that the buffers are also at room temperature.* *Note: Use our spin column protocol with spin columns product code sct-10 for easy elution. The use of spin columns ensure a minimal loss off the affinity resins during washing.* **(2)** Elution with SDS-Sample buffer (Laemmli): **Resuspend PARP1-Trap_A beads in 100 µl 2x SDS-sample buffer by pipetting up and down.** Make sure that all of the PARP1-Trap_A beads are resuspended. **Boil resuspended PARP1-Trap_A beads for 10 min at 95°C** to dissociate immune complexes from beads. PARP1-Trap_A beads can be collected by centrifugation at 2.500x g for 1 min at room temperature and SDS-PAGE is performed with the supernatant. **(3)** Elution with 8 M Urea: **Resuspend the beads in 50–100 µl 8 M Urea solution by pipetting up and down.** Make sure that all PARP1-Trap_A beads are resuspended. **Shake at 700 rpm for 5 min at room temperature. Then centrifuge at 2.500x g for 2 min at RT. Transfer the supernatant to a new tube.** To increase elution efficiency this step can be repeated.
Sample Preparation for Immunoblot Analysis	Add 50 µl 2x SDS-sample buffer to the collected samples from step 4 (Input) and step 8 (Flow Through). Incubate the samples for 10 min at 95°C. Spin down the sample before applying to gel.

Optional: Protocol for immunoprecipitation and elution of proteins from PARP1-Trap_A using Spin Columns

Note: spin columns (product code sct-10) are not included

Steps 1-5 describe the preparation of mammalian cell lysate.
For other types of cells we recommend using 0.5 – 1 mg of protein extract and start the protocol with Step 6.

Harvest mammalian cells	*Note: If you want to make an IP from other cell types like yeast, plants, etc. please use your own protocol for cell lysis and equivalent lysis buffer.* **1.** For one immunoprecipitation reaction the use of ~10^6 - 10^7 cells (approx. one 10-cm dish) expressing a protein of interest is recommended. **2.** To harvest adherent cells, aspirate growth medium, add 1 ml ice-cold PBS to cells and scrape cells from dish. Transfer cells to a pre-cooled tube, spin at 500 g for 3 min at +4°C and discard supernatant. Wash cell pellet twice with ice-cold PBS: Add 500 µl ice-cold PBS, gently resuspend the cells, centrifuge at 500 g for 3 min at 4°C. Carefully remove supernatant and discard. Repeat wash step twice. *Note: The cell pellet can be store for long term at -80°C.*
Lyse cells	**3. Resuspend cell pellet in 200 µl ice-cold lysis buffer by pipetting.** Supplement lysis buffer with protease inhibitors and DNase (not included). *Optional for nuclear/chromatin proteins: Use RIPA buffer supplemented with 1 mg/ml DNase, 2.5 mM $MgCl_2$ and protease inhibitors (not included).* **5. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.** **6. Centrifuge cell lysate at 20.000x g for 10 min at +4°C. Transfer supernatant (lysate) to a pre-cooled tube and add 300 µl 1x wash/dilution buffer. Discard pellet with cell debris.** If required, save 50 µl of the diluted lysate for immunoblot analysis (Input). *Note: Add 40 ml H_2O to 5x wash/dilution buffer before first use. It is 5 times concentrated!!* *Optional: Add protease inhibitors (not included) to 1x wash/ dilution buffer.* *Note: At this point cell lysate can be stored for long term at -80°C.*
Equilibrate beads	**7.** Beads can be equilibrated during incubation step of lysis procedure. **Remove the upper screw cap of a new spin column and snap of the tip from the bottom** (Keep cap and bottom plug!). **Place the spin column in a 2 ml tube**. **Pipette 500 µl ice-cold 1x wash/dilution buffer in the spin column**. *Note: Add 40 ml H_2O to 5x wash/dilution buffer before first use. It is 5 times concentrated!!* **8. Vortex PARP1-Trap_A beads intensively and directly pipette 25 µl bead slurry into the 1x wash/dilution buffer in the spin column. Pipette up and down a few times.** *Note: The slurry is more efficiently drawn into a wide bore pipette tip. We suggest clipping a little off the end of a regular tip to mimic the benefit of a wide bore tip. It is important to thoroughly resuspend the Nano-Trap-beads slurry by vortexing.* **9. Centrifuge at 100x g for 5-10 sec. Discard flow-through and repeat wash step twice**. The beads remain on top of the membrane. **10. Close column with the bottom plug**.

Bind Protein	**11. Add diluted lysate (from step 6) to equilibrated PARP1-Trap_A beads (from step 9).** Screw on upper cap. **Tumble end-over-end for 30 min at 4°C.** **12. Remove the bottom plug from the spin column and loose top cap. Place column in a new 2 ml tube. Centrifuge at 100x g for 5-10 sec**. If required, save 50 µl flow-through for immunoblot analysis (Flow Through). **Discard remaining flow-through.**
Wash Beads	**13. Add 500 µl ice-cold 1x wash/dilution buffer on top of the membrane to resuspend the PARP1-Trap_A beads. Centrifuge at 100x g for 5-10 sec. Discard flow-through and repeat wash steps twice.** *Optional: The salt concentration could be increased in the second washing step up to 500 mM.* **14. Close column with the bottom plug and place in a new tube.**
Elute Protein	**Depending on your downstream application different elution methods are possible:** **(1)** Elution with Glycine- Elution Buffer: **Add 50 µl Elution buffer to PARP1-Trap_A beads. Pipette beads up and down for 30 sec.** Make sure that all of the PARP1-Trap_A beads are resuspended. Close screw cap on top. **Remove bottom plug of the spin column and pipette 5 µl 1M Tris base pH 10.4 in the 2 ml tube for an immediate neutralization. Centrifuge at 100x g for 30-60 sec**. To increase elution efficiency this step can be repeated. *Note: It is important that the elution step and the neutralization is done at room temperature and that the buffer is also at room temperature.* **(2)** Elution with SDS-Sample buffer (Laemmli): **Resuspend PARP1-Trap_A beads in 100 µl 1x wash/dilution buffer. Then transfer diluted beads to a new tube. Centrifuge at 1000x g for 30-60 sec** to collect beads and remove was/dilution buffer (supernatant). **Add 100 µl 2x SDS-sample buffer by pipetting up and down.** Make sure that all of the PARP1-Trap_A beads are resuspended. **Boil resuspended PARP1-Trap_A beads for 10 min at 95°C** to dissociate immune complexes from beads. Beads can be collected by centrifugation at 2.500x g for 1 min at room temperature and SDS-PAGE is performed with the supernatant. **(3)** Elution with 8 M Urea: **Resuspend the beads in 50–100 µl 8 M Urea solution by pipetting up and down.** Make sure that all PARP1-Trap_A beads are resuspended. Close screw cap on top. **Shake at 700 rpm for 5 min at room temperature. Remove bottom plug of the spin column and centrifuge at 1000x g for 30-60 sec**. To increase elution efficiency this step can be repeated.
Sample Preparation for Immunoblot Analysis	Add 50 µl 2x SDS-sample buffer to the collected samples from step 6 (Input) and step 12 (Flow Through). Incubate the samples for 10 min at 95°C. Spin down the sample before applying to gel.

Support Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com*