

PolySUMO Capture & Release Kit (Cat. # J4410)

This kit is sufficient for purification of polysumoylated proteins from 100 mg or 20 X 5 mg cell or tissue extracts at a concentration of 5 mg/ml.

Description

The PolySUMO Capture & Release Kit is designed to rapidly enrich polysumoylated proteins from cell or tissue extracts within 6 hours according to a published method (reference 1) with modifications. This approach uses the SIM domains of RNF4 to capture polysumoylated proteins, which are subsequently released by incubating with a competitive peptide that binds SIM.

Components

Component	Stock	Quantity
• GST-4XSIM	4 mg/ml	1 ml
• GST	4 mg/ml	1.2 ml
• 2X Elution Peptide	2 X	0.45 ml
• Glutathione Agarose Resin	50% slurry	1.5 ml
• Iodoacetamine (IAA)	25 mg	1 Tube
• 20X Cell Lysis Buffer	20 X	2 X 1.0 ml
• NaCl	1 M	4 X 1.25 ml

Components in 20X lysis buffer

- 400 mM Tris, pH 7.6 at 4 °C
- 40 mM 2-mercaptoethanol (β ME)

Additional reagents required (not included in the kit)

- glycerol and MilliQ water

To make 50X IAA

- Reconstitute the supplied IAA powder into 270 μ l MilliQ H₂O or your buffer to make a stock solution of 500 mM (50X).

To make 20 mL 1X Cell Lysis Buffer

• 20X Cell Lysis Buffer	1 ml
• 1 M NaCl	3 ml
• 50X IAA	0.4 ml
• Glycerol (not provided)	2 ml
• MilliQ water	13.6 ml



Protocol

The following protocol has been used to test the kit for purification polysumoylated proteins from 50 mg HEK 293T whole cell lysates, optimizing the purification conditions may be necessary for your own experiments. Check the reference below to see additional purification options, such as adding protease inhibitor cocktail in cell lysates and including non-denaturing detergents in lysis/wash/elution buffers.

 **All purification steps are operated at 4°C!**

[Prepare and Lyse cells]

1. Sixteen dishes (150 mm) of HEK 293 cells were grown in DMEM supplemented with 10% fetal bovine serum to approximately 95% confluence. Cells were harvested and kept in a 50 ml conical tube. Cells can be frozen in -80 °C freezer for future use.
2. Resuspend the cell pellet in 24 ml cell lysis buffer (20 mM Tris, pH 7.6, 150 mM NaCl, 2 mM β ME, 10 mM IAA, 10% Glycerol). Briefly sonicate cells using a 550 Sonic Dismembrator (Fisher Scientific). Settings: power output: 2, 20 seconds/time for three times, rest 2 min between sonication.
3. Ultracentrifuge the cell lysates using a 70.1TI rotor (Beckman) at 36,000 rpm for 45min.
4. Carefully transfer the supernatant (avoid the lipids on the top) to a new 50 ml conical tube. We use a 10 ml syringe with a 21-gauge needle to take the supernatant. Repeat step 3 to further remove lipids if necessary.
5. Measure the cell lysate concentration using the Bradford assay. We obtained a total of 106 mg cell extracts.

[Bind polysumoylated proteins to GST-4XSIM]

6. Split the cell extracts in step 5 equally into two parts, 12 ml each in a 15 ml conical tube. In the control reaction, add GST to 0.2 mg/ml; in another tube, add GST-4XSIM to 0.2 mg/ml. Add 200 μ L glutathione agarose resin (net resin volume) into each tube. Mildly rotate the mixtures for 3 hours using a LABQUAKE rotator. More or less GST or GST-4XSIM may be used depending on your cell lysate concentration.

[Wash Glutathione agarose resin]

7. After incubation, centrifuge the mixtures using an IEC Centra CL3R centrifuge at 1,000 Xg for 5 min, discard the supernatant.
8. Add 0.5 ml washing buffer (lysis buffer plus 0.15M NaCl) to the conical tube, transfer resins to a 1.7 ml microcentrifuge tube (try to transfer all resins, multiple rinses and transfers are usually necessary).
9. Rotate the mixtures in step 8 for 3 min using a LABQUAKE rotator, then centrifuge using a desktop centrifuge at 1,000 Xg for 5 min, discard the supernatant.
10. Wash the resins two more times using 1.5 ml washing buffer. Discard the supernatant after centrifugation.

[Elute polysumoylated proteins]

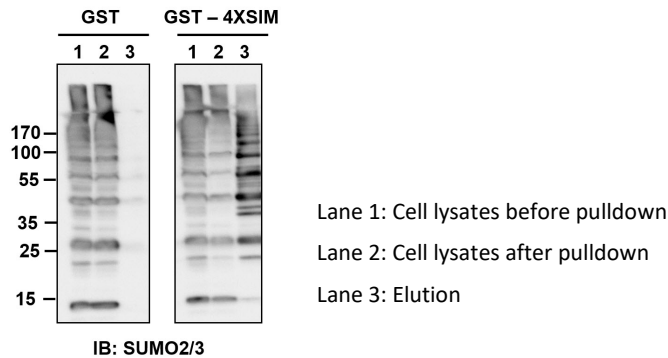
11. Resuspend the washed glutathione resins into 100 μ l cell lysis buffer, add 100 μ l 2X elution peptide (provided in 40 mM Tris, pH7.6 at 4 °C, 250 mM NaCl, 10% glycerol). Mildly rotate or rock the mixtures for 60 min.



12. Centrifuge the mixtures in step 11 using a desktop centrifuge at 1,000 Xg for 5 min. Carefully transfer the supernatant into a new 0.65 ml microcentrifuge tube. Rinse the glutathione agarose resins with 200 μ l lysis buffer and repeat the centrifugation step, combine the supernatant with the first elution.

[Quantification]

13. 20 μ l cell lysates before and after GST or GST-4XSIM binding and 5 μ l eluate were separated on a SDS-PAGE and immunoblotted with an antibody recognizing SUMO2 and SUMO3 (see attached Figure).



[Reference]

1. Bruderer R, et al. Purification and identification of endogenous polySUMO conjugates. (2011) EMBO Rep. 12(2):142-8.

