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# Unconjugated (Free) Polyubiquitin Chain Capture Kit

## (Cat. # J4420)

***Description***

The Unconjugated (Free) Polyubiquitin Chain Capture Kit is designed to rapidly enrich free polyUb chains in cell or tissue extracts within 6 hours. This approach uses the N-terminal ZnF-UBP domain of the deubiquitinating enzyme USP5 to specifically capture free polyUb chains. The ZnF-UBP domain of USP5 binds the C-terminal diglycine motif in Ub and free Ub chains, it does not bind polyUb chains conjugated on substrate proteins.

***Components***

| Component | Stock Concentration | Quantity |
|---|---|---|
| • GST-ZNF-UBP | 4 mg/ml | 0.5 ml |
| • GST | 4 mg/ml | 0.5 ml |
| • Iodoacetamine (IAA) | 25 mg | 1 Tube |
| • Glutathione (reduced) | 100 mg | 1 Tube |
| • Glutathione Agarose Resin | 50% slurry | 1.5 ml |

## *Notes*

1. This kit is recommended for enrichment of free polyUb chains in 50 mg cell or tissue lysates. We recommend using 0.2 mg GST-ZNF-UBP per 5 mg whole cell or tissue lysates.
2. Reconstitute the supplied IAA powder into 270 µl distilled $H_2O$ or your buffer to make a 100X stock solution of 500 mM.
3. Dissolve 100 mg glutathione (reduced) into 30 mL elution buffer. Use ~150 µl 2 M NaOH to adjust pH to neutral.

***Protocol***

The following protocol has been used to test the kit for purification of free polyUb chains in 10 mg HeLa cell lysates, optimizing the purification conditions may be necessary for your own experiments.

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

**[Prepare and Lyse cells]**

1. 7 dishes (100 mm) of HeLa cells were grown in DMEM supplemented with 10% fetal bovine serum to approximately 95% confluence. Cells were harvested and kept in a 15 ml conical tube. Cells can be frozen in -80 °C freezer for future use.

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2. Resuspend the cell pellet in 5 ml lysis buffer (20 mM Tris, pH 7.2, 150 mM NaCl, 2 mM βME, 5 mM IAA, 1X protease inhibitor cocktail (Roche) and 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 sonications.
3. Ultracentrifuge the cell lysates using a 70.1TI rotor (Beckman) at 36,000 rpm for 45min.
4. Carefully transfer the supernatants (avoid the lipids on the top) to a new 15 ml conical tubes. We use a 10 ml syringe with a 21 gauge needle to take the supernatant. Recovered 4.5 ml supernatants.
5. Measure the supernatants concentration using the Bradford assay. Obtained a total of 24 mg cell extracts.

**[Bind polysomolyated proteins GST-4XSIM]**

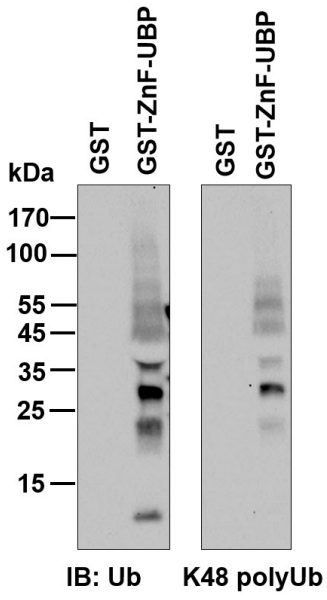
6. Split the cell extracts in step 5 into two copies, 1.8 ml each in a 2.2 ml centrifuge tube. In the control reaction, add GST to 0.2 mg/ml; in another tube, add GST-ZnF-UBP to 0.2 mg/ml. Add 75 µL Glutathione resin (net resin volume and washed three time using lysis buffer) into each tube. Mildly rotate the mixtures for 3 hours using a LABQUAKE rotator. More or less GST or GST-ZnF-UBP may be used depending on your cell extract concentration.

**[Wash Glutathione resin]**

7. After incubation, centrifuge the mixtures using a desktop centrifuge at 500 x*g* for 5 min, discard the supernatants.
8. Add 1.5 ml lysis buffer to each tube, rotate the mixtures for 5 min using a LABQUAKE rotator, then centrifuge using a desktop centrifuge at 500 x*g* for 3 min, discard the supernatants.
9. Repeat the washing in step 8 one more time. Discard the supernatants after centrifugation (careful not to lose glutathione resins).

**[Elute the bound free polyUb chains]**

10. Elute proteins using 200 µl buffer containing 10 mM glutathione. Pellet down the resin by centrifugation. GST-ZnF-UBP and its bound proteins will be in the supernatants. Bound proteins can be analyzed by immunoblotting or mass spectrometry.




10 µl GST-bound or GST-ZnF-UBP-bound proteins were separated on SDS-PAGEs and immunoblotted with antibodies again Ub or K48-linked polyUb chains.