

K29/K33 Polyubiquitin Chain Capture Kit (Cat. # J4470)

Description

This kit is designed for enrichment of cellular proteins conjugated with lysine 29/33-linked polyubiquitin chains using GST-TRABID (ZNF1). Polyubiquitinated proteins bound on GST-TRABID (ZNF1) can be precipitated using glutathione resin and eluted by a buffer containing 10 mM glutathione. Enriched polyubiquitinated proteins can be assayed by immunoblotting or mass spectrometry.

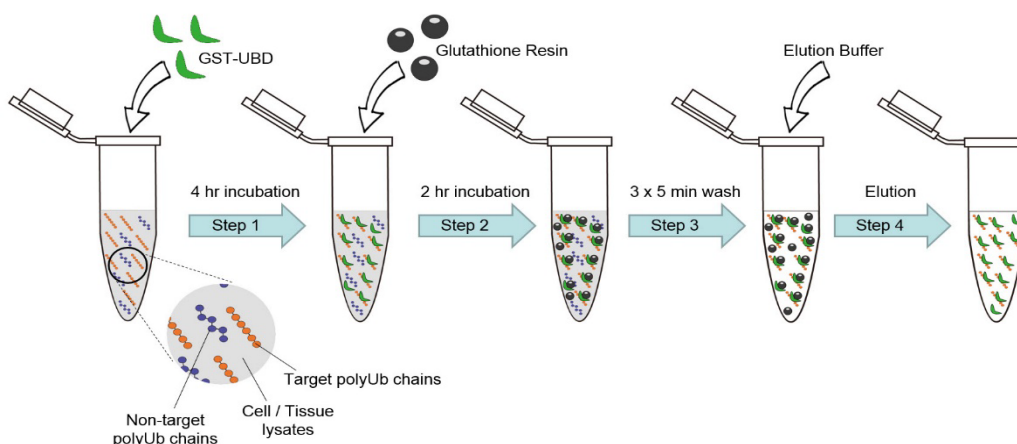
Components

Component	Stock Concentration	Quantity
• GST-TRABID (ZNF1)	2 mg/ml	1 ml
• GST	4 mg/ml	0.5 ml
• Iodoacetamide (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 proteins conjugated with lysine 29/33 polyubiquitin chains in 50 mg cell or tissue lysates. We recommend using 0.2 mg GST-TRABID (ZNF1) per 5 mg whole cell or tissue lysates.
2. Reconstitute the supplied IAA powder into 270 μ l distilled H₂O or your buffer to make a stock solution of 500 mM.
3. Dissolve 100 mg glutathione (reduced) into 30 mL elution buffer. Use \sim 150 μ l 2 N NaOH to adjust pH back to neutral.

A schematic illustrating enrichment of polyUb chains



Protocol

A procedure for capturing K29/K33 polyubiquitinated proteins in cell or tissue lysates (optimization may be required)





All purification steps are operated at 4°C!

- 1) Prepare whole cell or tissue lysates with a total protein concentration of 2-5mg/ml. Cell/tissue debris should be removed by high speed centrifugation. Protease inhibitors (not included) and 5-10 mM iodoacetamide (a cysteine protease inhibitor that blocks most deubiquitinating enzymes) can be added in the lysis buffer to block protease digestion and deubiquitination in the purification process.
- 2) We recommend adding 0.2 mg GST-TRABID (ZNF1) or GST (a negative control) per 5 mg whole cell or tissue lysates. Users can scale up or down according to your experimental needs. Optimization may be required. The mixtures can be incubated with slow rotation at 4 °C for 4-16 hours.
- 3) Wash 100 µl glutathione resin slurry (75 µL net resin) using the lysis buffer, then add the resin to the mixtures in step 2 for a 2-hour incubation with slow rotation. After incubation, glutathione resin can be pelleted down by centrifugation at 750 Xg for 5 min. Discard the supernatants or save as a control when analyzing samples. If necessary, transfer the resin to a 0.65 ml microcentrifuge tube for the following wash/elution steps.
- 4) Wash glutathione resin three times. Use 0.5 ml buffer containing 300-750 mM NaCl and rotate the mixtures 2-3 min for each wash. Pellet down the resin after each wash by centrifugation as described above, discard the supernatants or save as a control when analyzing samples.
- 5) Elute proteins using 200 µl buffer containing 10 mM glutathione. Pellet down the resin by centrifugation. GST-TRABID (ZNF1) and its bound proteins will be in the supernatants. Bound proteins can be analyzed by immunoblotting or mass spectrometry.

In our experiments, we resuspended 293 cells from 20 dishes of 10 cm plates into 10 ml lysis buffer containing 50 mM Tris, pH 7.6, 100 mM NaCl, 2 mM β-ME, 10% glycerol, 10 mM IAA and 1X protease inhibitor cocktail (Roche). Cells were lysed by brief sonication and the mixtures were ultracentrifuged at 36,000 rpm for 45 min using a Ti70.1 rotor. The resulting clear supernatant has a protein concentration of 10.1 mg/ml as determined by the Bradford assay. Other procedures were the same as listed above.

