

# Linear Polyubiquitin Chain Capture Kit (Cat. # J4450)

## Description

This kit is designed for enrichment of cellular proteins conjugated with linear polyubiquitin chains using GST-NEMO (UBAN). Polyubiquitinated proteins bound on GST-NEMO (UBAN) can be precipitated using glutathione resin and eluted by a buffer containing 10 mM glutathione. Polyubiquitinated proteins can be assayed by immunoblotting or mass spectrometry.

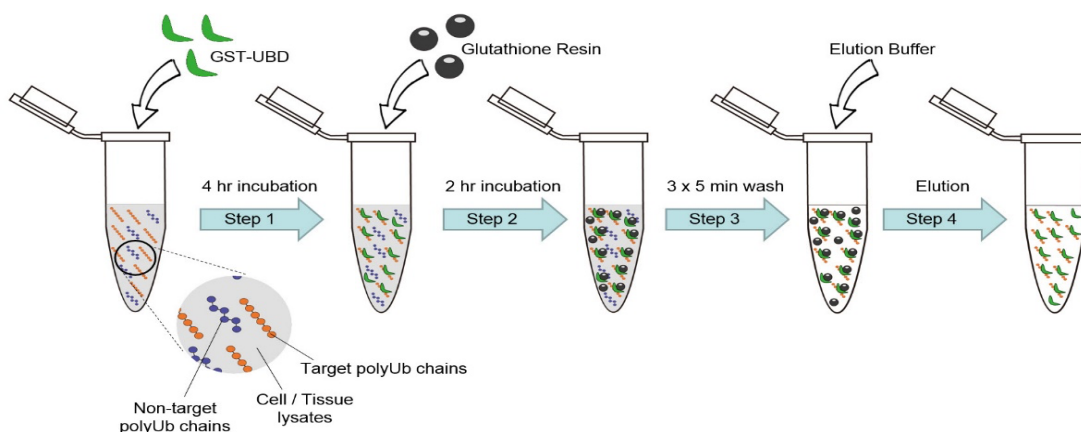
## Components

Component	Stock Concentration	Quantity
• GST-NEMO (UBAN)	4 mg/ml	0.5 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 linear polyubiquitin chains in 50 mg cell or tissue lysates. We recommend using 0.2 mg GST-NEMO (UBAN) per 5 mg lysates.
2. Reconstitute the supplied IAA powder into 270  $\mu$ l distilled H<sub>2</sub>O or your buffer to make a 100X stock solution of 500 mM.
3. Dissolve 100 mg glutathione (reduced) into 30 mL elution buffer. Use  $\sim$ 150  $\mu$ l 2 M NaOH to adjust pH back to neutral.

### A schematic illustrating enrichment of polyUb chains



## Protocol

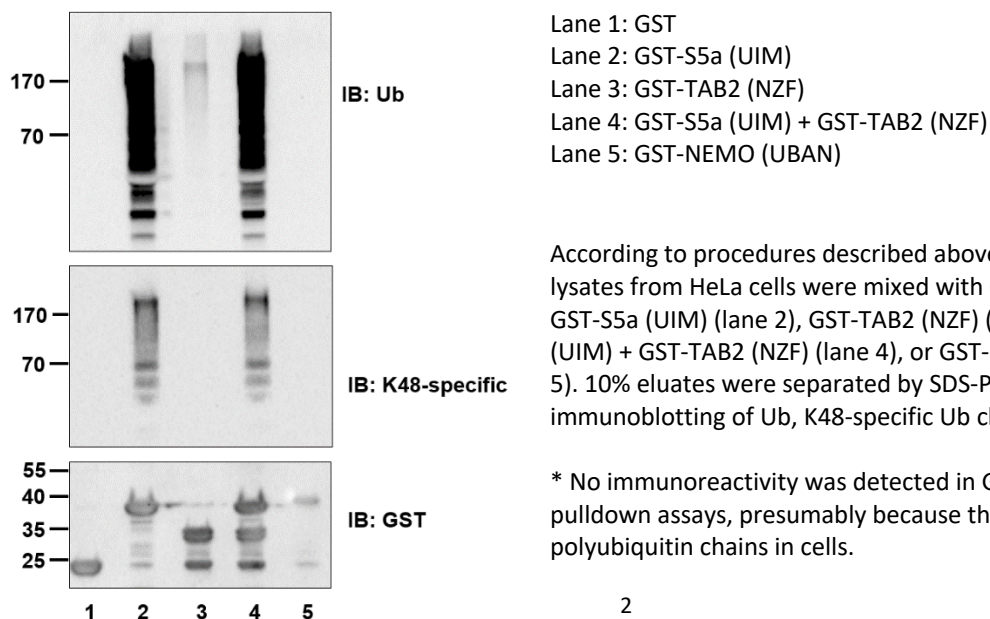
A procedure for capturing linear polyubiquitin chain in cell or tissue lysates (optimization may be required)



 **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 mM iodoacetamide (a cysteine protease inhibitor that inhibits 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-NEMO (UBAN) 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 150  $\mu$ l glutathione resin slurry (75  $\mu$ l net resin) three times using the lysis buffer, then add the resin to the mixtures in step 2 for a 2 hours 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 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  $\mu$ l buffer containing 10 mM glutathione (not supplied). Pellet down the resin by centrifugation. GST-NEMO (UBAN) and its bound proteins will be in the supernatants. Bound proteins can be analyzed by immunoblotting or mass spectrometry.

In our experiments, we suspended HeLa cells from 14 dishes of 10 cm plates into 6 ml lysis buffer containing 50 mM Tris, pH 7.6, 150 mM NaCl, 2 mM  $\beta$ ME, 10% glycerol, 5 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 7.6 mg/ml determined by the Bradford assay. Other procedures were the same as listed above.



According to procedures described above, 5 mg whole cell lysates from HeLa cells were mixed with 0.2 mg GST (lane 1), GST-S5a (UIM) (lane 2), GST-TAB2 (NZF) (lane 3), GST-S5a (UIM) + GST-TAB2 (NZF) (lane 4), or GST-NEMO (UBAN) (lane 5). 10% eluates were separated by SDS-PAGE, followed by immunoblotting of Ub, K48-specific Ub chains and GST.

\* No immunoreactivity was detected in GST-NEMO (UBAN) pulldown assays, presumably because there is little linear polyubiquitin chains in cells.

