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K63 polyUb Chain-binding Protein Identification / Validation Kit (Cat. # J2510)

* This kit is designed for two purposes: 1) identification of proteins that specially bind K63 polyUb chains in whole cell or tissue lysates; 2) validation of direct interactions between K63 polyUb chains and their binding proteins.

Description

The non-cleavable 6xHis-tagged K63 polyUb chains $_{(2\text{-}4)}$ are resistant of deubiquitination when incubating with whole cell or tissue lysates, thus allowing maximally capture proteins that specifically bind K63 polyUb chains. After binding, the non-cleavable K63 polyUb chains and the binding proteins can be enriched by Nickel *XPure* Agarose Resin (included). The bound proteins and the non-cleavable K63 polyUb chains can be eluted from Ni resin using a buffer containing 250 mM imidazole (included) or by incubating with thrombin (not included). Either of the elution method can significantly reduce the amounts of non-specific binding proteins when compared with elution using SDS sample buffer.

Components

Component	Quantity	Storage upon receiving
6xHis-Non-cleavable K63 polyUb chain $_{(2\text{-}4)}$	100 µg	-80 °C
6xHis-Ubiquitin	100 µg	-80 °C
Nickel *XPure* Agarose Resin (suspended in 50% slurry)	500 µl	2-8 °C
4 M Imidazole	500 µl	-80 °C
Iodoacetamide (IAA)	25 mg	-80 °C

Note

Reconstitute the supplied Iodoacetamide powder into 270 µL distilled water or your buffer to make a 500 mM stock solution

Procedure

A procedure for capturing K63 polyUb binding proteins in cell or tissue lysates (optimization may be required)

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 deubiquitinating enzyme inhibitor) can be added in the lysis buffer to block protease digestion and deubiquitination in the purification process.

Important note: add 15 mM imidazole in the lysis buffer to reduce non-specific binding on Ni resin in step 3.

2) We recommend adding 100 µg 6xHis non-cleavable K63 polyUb chains $_{(2\text{-}4)}$ or 6xHis-Ubiquitin (a negative control) with 5-10 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 200 µl Ni resin slurry (100 µL net Ni resin) using the lysis buffer, then add Ni resin to the mixtures for 1-2 hour further slow rotation. After incubation, Ni 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 Ni resin to a 0.65 ml microcentrifuge tube for the following wash/elution steps.

4) Wash Ni resin three times. Use 0.5 ml buffer containing 30 mM imidazole and rotate the mixtures 2-3 min for each wash. Pellet down Ni resin after each wash by centrifugation as described above, discard the supernatants or save as a control when analyzing samples.

5) Elute proteins using 100-200 µl buffer containing 250 mM imidazole. Pellet down Ni resin by centrifugation. Non-cleavable K63 polyUb chains and their binding proteins will be in the supernatants. Bound proteins can be analyzed by immunoblotting or mass spectrometry.