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EZEnrich™ Polyubiquitin Beads

03/14

Store at 4°C. Do not freeze.

Cat. No.: 6568-300 **300 µl of the slurry of beads**

Salient Features:

Binding Capacity:	~ 2.06 µg/20 µl of slurry.
Bead content:	300 µl of 50% slurry of UIM-UBA Sepharose Beads in PBS with 0.1% Sodium Azide.
Total Capacity:	15 enrichments with 20 µl beads each.
Specificity:	The EZEnrich™ Polyubiquitin Beads bind polymers of ubiquitin containing at least 4 ubiquitin subunits.

Recommended complimentary products:

Cat # 6572-50 - Multipurpose Mini Spin Columns. These columns can be used for the enrichment.
Cat # K6570-30 - EZ Extract™ Polyubiquitin Buffer Kit. This kit can be used for preparation of lysis buffer required for sample preparation.

Description:

BioVision's EZEnrich™ Polyubiquitin Beads are ideal for isolating or enriching polyubiquitin modified proteins from biological samples. Ubiquitin is a highly conserved 76-amino acid protein. It can be conjugated via its C-terminus to the amine groups of lysine residues on target proteins. This conjunction is referred to as monoubiquitylation. Additional ubiquitin moieties can be subsequently conjugated to this initial ubiquitin, utilizing any one of the seven lysine residues on the surface of ubiquitin. The formation of these ubiquitin chains is referred to as polyubiquitylation. Different types of polyubiquitin chains can form, depending on the internal lysine residue used for this conjugation. These polyubiquitin chains further can attach to proteins post-translationally and aid in numerous downstream activities like proteasome-mediated proteolysis, autophagy, DNA damage tolerance, inflammation, apoptosis, signal transduction etc. Several classes of Ubiquitin interacting proteins help in mediating these downstream effects. Ubiquitin Interacting Motifs (UIM) and Ubiquitin Associated Domains (UBA) are two large classes of such protein domains which strongly interact with polyubiquitin chains.
We have developed an UIM-UBA chimeric protein from the UIM and UBA domains. Both these proteins are well-characterized for their high-affinity interaction with different types of polyubiquitin chains. The UIM-UBA protein is covalently bound to our sepharose beads (Cat # 6565) leading to a high binding affinity. These UIM-UBA sepharose beads are ideal for convenient and efficient enrichment of poly-ubiquitinated proteins, with minimal contamination.

Applications:

- Isolation/enrichment of poly-ubiquitinated proteins from complex samples including tissue homogenates, whole cell lysates, subcellular organelles like mitochondria, endoplasmic reticulum, etc, for multiple downstream applications like proteomic studies, protein ubiquitination status tests etc.
- Recovery of poly-ubiquitinated proteins from *in vitro* ubiquitination reactions.
- Subjective quantitation of the differences in the ubiquitination of different target proteins.

User Supplied Reagents and Equipments:

- Lysis Buffer – Phosphate Buffered Saline (PBS) with 0.1% Triton X-100, 2 mM N-ethylmaleimide (NEM) and protease inhibitor cocktail (K271-500).
- This protocol is compatible with our EZ Extract™ Polyubiquitin Buffer Kit (Cat # K6570).

Enrichment Procedure for Poly-ubiquitinated Proteins

The following protocol uses 20 µl beads suspension to enrich polyubiquitins from approximately 150 µg-600 µg sample (protein concentration at 0.25 mg/ml to 1.0 mg/ml). Scale up accordingly if you have a larger sample size. The enrichment procedure may be carried out as described below, using either our Multipurpose Mini Spin Columns (Cat. # 6572) **(A)** or a batch method **(B)**.

A. Enrichment Procedure: Multipurpose Mini Spin Columns (Cat. # 6572)

A1.Sample: Prepare samples using our EZ Extract™ Polyubiquitin Buffer Kit (Cat # K6570) or the Lysis Buffer described above (it is recommended to include 2 mM N-ethylmaleimide (NEM) in lysis buffer to minimize de-ubiquitination during the whole process). The maximal compatible sample volume is ~ 600 µl.

A2. Gently resuspend the beads and then pipette (cut pipette tip) 20 µl of the bead suspension into an empty mini spin column.

A3. Transfer samples into the spin column loaded with beads and close the cap securely.

A4. Mix the sample and beads gently end-over-end for 1 hr at room temperature or 2 hrs at 4˚C.

A5. Snap off and save the bottom closure (the distal end works to plug column during Elution step) and slightly loosen the top cap.

A6. Place the column in a 2.0 ml microcentrifuge tube, centrifuge at 150 g x 1 min, collect and keep flow-through for later analysis.

A7. Place the column back in a tube, add 500 µl Lysis Buffer, centrifuge at 150 g x 1 min and discard the solution.

A8. Repeat Step A7 twice for 2 more washing.

A9. Use the plug saved at step A5 to close the bottom of column securely. Add 40 µl of 1x SDS-PAGE loading buffer to the column, cap the top of column tightly and gently shake to mix the beads and solution.

A10. Leave the column in a 1.5 ml microcentrifuge tube, and heat the tube in a 100˚C heating block for 5 min.

A11. Slightly open the top cap of column first, then remove the bottom plug, and place the column into a clean 1.5 ml microcentrifuge tube. Centrifuge at 1,500 g for 30 seconds to collect the eluate for SDS-PAGE / Western blot analysis.

B. Polyubiquitin Enrichment procedure: Batch Method

B1.Sample: Prepare samples using our EZ Extract™ Polyubiquitin Buffer Kit (Cat # K6570) or the Lysis Buffer described above (it is recommended to include 2 mM N-ethylmaleimide (NEM) in lysis buffer to minimize de-ubiquitination during the whole process).

B2. Gently resuspend beads and then pipette (cut pipette tip) 20 µl of bead suspension into a 1.5 ml microcentrifuge tube.

B3. Transfer samples into the 1.5 ml microcentrifuge tube loaded with beads and close the lid securely.

B4. Mix the sample and beads gently end-over-end for 1 hr at room temperature or 2 hrs at 4˚C.

B5. Centrifuge at 150 g x 1 min to pellet down the beads and remove the solution (keep for later analysis).

B7. Add 500 µl Lysis Buffer to the 1.5 ml microcentrifuge tube, close tube and invert 6 times to wash the beads

B8. Centrifuge at 150 g x 1 min and discard the solution.

B9. Repeat Steps B7 & B8 twice for 2 more washes; remove the residual solution as much as possible.

B10. Add 40 µl 1x SDS-PAGE loading buffer into the 1.5 ml microcentrifuge tube, close tube tightly and mix well.

B11. Heat the 1.5 ml microcentrifuge tube in a 100˚C heating block for 5 min.

A12. Centrifuge the 1.5 ml microcentrifuge tube at 10,000 g for 30 seconds to collect the supernatant for SDS-PAGE / Western blot analysis.

Figure 1

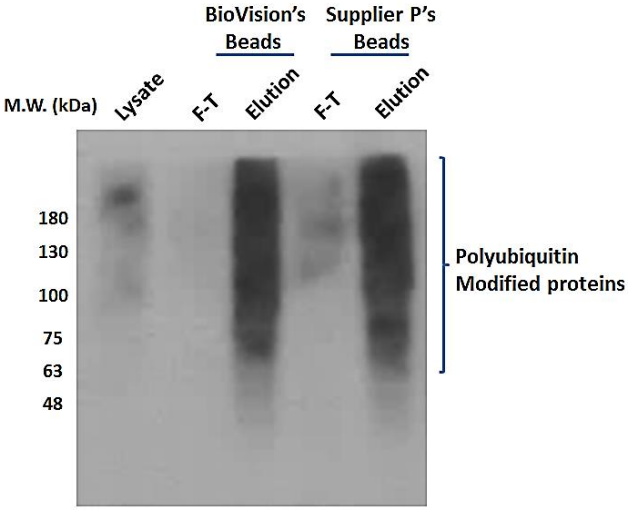

Figure 1. EZEnrich™ Polyubiquitin Beads for capturing polyubiquitin conjugates in cell lysates. Jurkat cells treated with MG132 (5 µM for 20 min), were lysed using our EZ Extract™ Polyubiquitin Buffer Kit (Cat # K6570) to obtain clear cell lysates. Protein concentration was adjusted to 1 µg/µl. The polyubiquitin modified proteins were enriched using the EZEnrich™ Polyubiquitin Beads (20 µl) with our Multipurpose Mini Spin Columns (Cat # 6572) from 150 µg of MG132 Jurkat cell lysate. A similar kit from Supplier P was used to enrich polyubiquitin conjugates, by following the manufacturer's protocol. The starting material (**Lysate**, 30 µg), flow-through (**F-T**, volume equivalent to that of cell lysate containing 30 µg proteins) and **Eluate** were analyzed using Western blot with a polyclonal ubiquitin antibody.
BioVision's EZEnrich™ Polyubiquitin Beads are more efficient in capturing polyubiquitin modified proteins in the eluate as evidenced by the quantitative removal of these proteins in the FT.

RELATED PRODUCTS:

EZ Extract™ Polyubiquitin Buffer Kit (Cat # K6570-30)
Multipurpose Mini Spin Columns (6572-50)
Ready-to-Use Ni-IDA Spin Purification Kit (K6567-25)
Hi-Bind™ Ni QR Agarose Beads (6562)
Benzonase Nuclease (Cat. #7680)
10K Spin Column (1997)
Ready-to-use Ni QR Agarose Beads Buffer Kit (K6563-3)
Protein G-Sepharose Column (6518)
Protein A/G-Sepharose Column (6528)
DiaEasy™ Dialyzer (250 µl) MWCO 6-8 kDa (K1020)
DiaEasy™ Dialyzer (250 µl) MWCO 25 kDa (K1022)
DiaEasy™ Dialyzer (800 µl) MWCO 3.5 kDa (K1018)
DiaEasy™ Dialyzer (3 ml) MWCO 3.5 kDa (K1012)
DiaEasy™ Dialyzer (3 ml) MWCO 12-14 kDa (K1014)
DiaEasy™ Dialyzer (3 ml) MWCO 50 kDa (K1016)

EZDetect™ Polyubiquitin Probe (6569-250)
Streptavidin-Sepharose Beads (6565-2, -5, -10)
Ready-to-use Ni QR Agarose Beads Buffer Kit (6563-3)
EZ-Desalt™ Spin Desalting Columns (6564-25)
Glutathione Sepharose (6555)
Protein A-Sepharose Column (6508)
Protein L-Sepharose Column (6538)
Protein A/G/L-Sepharose Column (6548)
DiaEasy™ Dialyzer (250 µl) MWCO 12-14 kDa (K1021)
DiaEasy™ Dialyzer (800 µl) MWCO 1 kDa (K1017)
DiaEasy™ Dialyzer (800 µl) MWCO 6-8 kDa (K1019)
DiaEasy™ Dialyzer (3 ml) MWCO 6-8 kDa (K1013)
DiaEasy™ Dialyzer (3 ml) MWCO 25 kDa (K1015)

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