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EZDetect™ Polyubiquitin Probe (Biotin conjugated)

03/14

Store at 4°C. Do not freeze.

Cat. No.: 6569-250 250 µl (100 µg) of Biotin-UIM-UBA protein (0.4 mg/ml) in PBS with 0.1% Azide.

Description:

BioVision's EZDetect™ Polyubiquitin Probe is ideal for detecting poly-ubiquitinated proteins in 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 EZDetect™ Polyubiquitin Probe is synthesized through biotinylation of UIM-UBA with Sulfo-NHS-LC-Biotin (Cat # 2326). This biotin-UIM-UBA can be used to detect poly-ubiquitinated proteins by decreasing the interference from free monomeric ubiquitin which is highly abundant in biological samples.

Applications:

- To detect the poly-ubiquitinated proteins in cell lysate/tissue homogenate using **Dot blot.**
- To detect the poly-ubiquitinated proteins in cell lysate/tissue homogenate using **Western blot**.
- In **Immunocytochemistry / Immunohistochemistry** studies to label / visualize intracellular poly-ubiquitinated proteins.

Dot Blot Procedure:

1. Lyse cells or homogenize tissue with our EZ Extract™ Polyubiquitin Buffer Kit (Cat # K6570) or any preferred lysis buffer with detergent, N-ethylmaleimide (NEM) and protease inhibitor cocktail (K271-500). Adjust the sample to the desired protein concentration. The recommended protein concentration is 1-5 µg/µl.

2. Prepare 1:2 serial dilutions of the samples. Include the lysis buffer only as the negative control sample. Label samples as A, B, C, D, E, F, G and H, with A for original / undiluted cell lysate, and H for Lysis Buffer (as negative control).

3. Specify the areas for the dot blot on a nitrocellulose membrane from A to H horizontally, and 1, 2 vertically, as shown below in

Figure 1

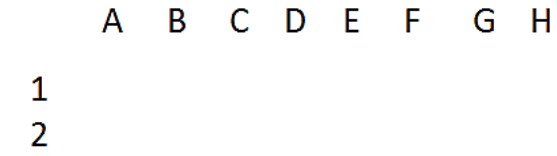
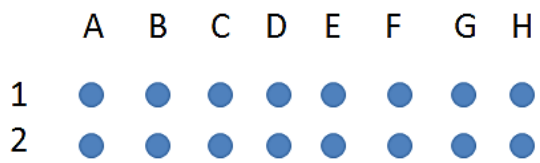


Figure 2



2.

4. Using a narrow-mouth pipette tip, slowly apply 1 µl of sample onto each spot on the nitrocellulose membrane as shown in **Figure 2.**

5. Air dry the nitrocellulose membrane for 60 min.

6. Block any non-specific binding sites by soaking the nitrocellulose membrane in 5% Non-fat milk in TBS/T for 30 min at RT.

7. Incubate the membrane with the diluted EZDetect™ Polyubiquitin Probe (1 µg/ml in TBS/T [2.5 µl in 1 ml of TBS/T]) for 60 min at RT with gentle shaking.

8. Wash the membrane three times with TBS/T (3 x 5 mins).

9. Incubate the membrane with Streptavidin-HRP (for optimal dilution, follow the manufacturer's recommendation) for 40 min at RT.

10. Wash three times with TBS/T (3 x 10 mins) and then rinse the membrane once with TBS.

11. Develop the membrane with ECL reagent for 1 min, and expose the X-ray film in a dark room. Try different exposure times to optimize the signal.

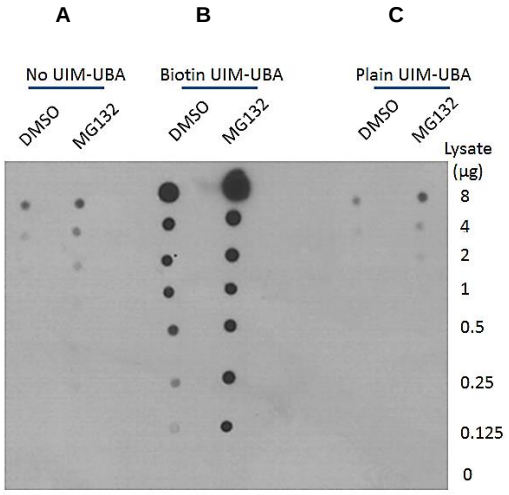
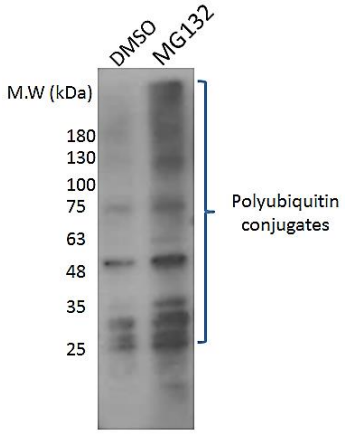


Figure 3. Dot Blot.
Jurkat cells were treated with DMSO or MG132 (5 µM for 20 min), and lysed to obtain clear cell lysates (8 µg/µl). The serially diluted samples were spotted on to a nitrocellulose membrane, for Dot-Blot detection using EZDetect™ Polyubiquitin Probe (1 µg/ml) in **Panel B**. Detection of the poly-ubiquitinated proteins by Biotin-UIM-UBA, especially in MG132 cell lysates validates Biotin-UIM-UBA as a selective and easy tool in assessing poly-ubiquitinated proteins. As negative controls, no UIM-UBA protein was used in **Panel A**, and non-biotinylated UIM-UBA was used in **Panel C**. Only minimal background signals were detected in both these panels.

Western Blot Procedure

1. Run a SDS-PAGE and transfer the proteins by following standard procedures.
2. Block any non-specific binding sites by soaking the nitrocellulose membrane in 5% Non-fat milk in TBS/T for 30 min at RT.
3. Incubate the membrane with the diluted EZDetect™ Polyubiquitin Probe (1 µg/ml in TBS/T) for 60 min at RT with gentle shaking.
4. Wash the membrane three times with TBS/T (3 x 5 mins).
5. Incubate the membrane with Streptavidin-HRP (for optimal dilution, follow the manufacturer's recommendation) for 40 min at RT.
6. Wash three times with TBS/T (3 x 10 mins) and then rinse the membrane once with TBS.
7. Develop the membrane with ECL reagent for 1 min, and expose the X-ray film in a dark room. Try different exposure times to optimize the signal.

Figure 4. Western blot application
Jurkat cells were treated with DMSO or MG132 (5 µM for 20 min) and lysed to obtain clear cell lysates. The lysates (30 µg proteins) were resolved on 4-20% SDS-PAGE. Following standard procedures of membrane transfer, and blocking, the blot was probed with Biotin-UIM-UBA (1 µg/ml, O/N at 4˚C). Incubation with Streptavidin-HRP of blot was followed by ECL detection. Inhibition of proteasome degradation by MG132 results in the intracellular accumulation of poly-ubiquitinated proteins. More poly-ubiquitinated proteins are detected in MG132 cell lysates, than in that of DMSO-treated cell lysate. This demonstrates the specificity of detection of only poly-ubiquitinated proteins with Biotin-UIM-UBA's in western blotting application.



RELATED PRODUCTS:

EZ Extract™ Polyubiquitin Buffer Kit (K6570-30)
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)
Multipurpose Mini Spin Columns (6572-50)
EZEnrich™ Polyubiquitin Beads (6568-100)
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)

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