

U-Blot[®] K- ϵ -GG Specific Antibody Conjugated Agarose Beads (WT9151B)

User Manual

General Information

Product name	U-Blot [®] K- ϵ -GG Specific Antibody Conjugated Agarose Beads
Product No.	WT9151B
Clonality	Recombinant monoclonal

Key Features

Target	K- ϵ -GG
Reactivity	Species Independent
Applications	IAC, IAP, IP
Isotype	IgG
Conjugate	Agarose Beads
Bead size	45–165 μ m (cross-linked 4 % agarose beads)

Storage Information

Storage	12 months from date of receipt / reconstitution, 2 to 8°C as supplied
Formulation	50% slurry in PBS pH7.4, 0.05% Proclin 300

Target Information

Background

Ubiquitination is a vital post-translational protein modification that extensively regulates diverse cellular biological activities. After cleavage during ubiquitination modification, a characteristic diglycine structure remains on lysine residues, namely the K- ϵ -GG motif. In proteomics research, specific antibodies are applied to enrich peptides carrying K- ϵ -GG modification, followed by peptide identification and quantification via mass spectrometry. This approach enables exploration of the functional mechanism of ubiquitination in physiological processes and its alteration during disease progression. Antibodies targeting the K- ϵ -GG motif serve as core research tools for ubiquitinomics. Combined with immunoaffinity enrichment and liquid chromatography-tandem mass spectrometry, they support large-scale qualitative and quantitative analysis of ubiquitinated proteins.

Protocol

Required buffer solutions

IP Wash Buffer 1: 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, supplemented with 0.5% NP-40 and 0.1% Triton X-100, pH adjusted to 8.0

IP Wash Buffer 2: 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH adjusted to 8.0

IP Wash Buffer 3: 50 mM Ammonium Bicarbonate solution

Immunoaffinity Procedures

- 1、Gently resuspend beads with a pipette. Vortexing is prohibited;
- 2、Transfer 50 μ L bead slurry into a centrifuge tube containing 0.5 mL pre-chilled PBS.
- 3、Centrifuge at $1000 \times g$ for 1 minute at 4 °C, discard supernatant and repeat washing twice.
- 4、Dissolve 2 mg desalted peptide sample in 1 mL PBS.

- 5、Centrifuge the sample solution at $12000 \times g$ for 10 minutes at 4°C to remove solid precipitates.
- 6、Add the clarified peptide solution to beads, incubate overnight with end-over-end rotation at 4°C .

Washing Process

- 1、Centrifuge at $1000 \times g$ for 1 minute at 4°C to pellet beads.
- 2、Add 0.5 mL Wash Buffer 1, invert the tube 15 times for thorough mixing, centrifuge and discard waste liquid. Repeat the process no less than twice.
- 3、Replace with 0.5 mL Wash Buffer 2, mix and centrifuge in the same manner, discard supernatant and repeat at least twice.
- 4、Add 0.5 mL Wash Buffer 3 finally, mix and centrifuge to remove impurities, repeat twice or more.

Peptide Elution Operation

- 1、Completely remove residual liquid in the tube.
- 2、Add 100 μL elution buffer containing 0.2% trifluoroacetic acid and fully resuspend beads.
- 3、Incubate for 10 minutes with end-over-end rotation at room temperature.
- 4、Separate liquid from beads by low-temperature centrifugation, transfer eluate into a new centrifuge tube.
- 5、Perform elution twice more and combine all collected eluates.
- 6、Desalt samples via C18 column, freeze-dry, reconstitute with loading buffer and proceed to mass spectrometry detection.

Precautions

- 1、Do not vortex beads throughout the experiment. Gently resuspend beads only by pipetting to protect agarose bead structure and antibody activity. Wide-bore pipette tips are recommended to avoid tip clogging.
- 2、The three wash buffers feature gradually reduced ionic strength and detergent content, which eliminates non-specific binding while preventing loss of target peptides. Thoroughly invert and mix samples in each washing step to ensure sufficient contact between beads and buffer.
- 3、Optimization Double elution combined with pooled eluates can effectively improve target peptide recovery rate. Strictly control pH value and TFA concentration of elution buffer to avoid interference with subsequent mass spectrometry analysis.

For Research Use Only. Not for Use in Diagnostic Procedures