PRODUCT INFORMATION

Ubiquitin Remnant (K-ε-GG) Immunoaffinity Sorbent

Item No. 34699

Overview and Properties

Contents: This vial contains 400 µl of CNBr-activated Sepharose 4B coupled to Diglycyl-Lysine Monoclonal Antibody (Clone GX41) (Item No. 21096). The product is supplied as a 50% slurry in PBS, pH 7.2, with 0.02% sodium azide.
Immunogen: Diglycine-modified histone peptide
Cross Reactivity: (+) Diglycyl-lysine moieties
Form: Liquid
Storage: 4°C (as supplied)
Stability: ≥1 year
Storage Buffer: PBS, pH 7.2, with 0.02% sodium azide
Clone: GX41
Host: Mouse
Isotype: IgG1
Applications: Suitable for immunoprecipitation (IP) (LC/MS certified), working concentration/dilution should be determined empirically.

Image

Identified Proteins (2,252)	Accession Number	Molecular Weight	Untreated Cells	MG132-Treated Cells
Ubiquitin-40S ribosomal protein S27a OS=Homo sapiens OX=9606 GN=RPS27A PE=1 SV=2	sp|P62979	18 kDa	145	198
Elongation factor 1-alpha 1 OS=Homo sapiens OX=9606 GN=EEF1A1 PE=1 SV=1	sp|P68104	50 kDa	10	10
Heat shock 70 kDa protein 1A OS=Homo sapiens OX=9606 GN=HSPA1A PE=1 SV=1	sp|P0DMV8	70 kDa	63	70
DNA-dependent protein kinase catalytic subunit OS=Homo sapiens OX=9606 GN=PRKDC PE=1 SV=3	sp|P78527	469 kDa	22	138
eIF-2-alpha kinase activator GCN1 OS=Homo sapiens OX=9606 GN=GCN1 PE=1 SV=7	sp|Q92616	293 kDa	5	134
Tubulin alpha-1B chain OS=Homo sapiens OX=9606 GN=TUBA1B PE=1 SV=1	sp|P68363	50 kDa	26	60
Heterogeneous nuclear ribonucleoproteins A2/B1 OS=Homo sapiens OX=9606 GN=HNRNPA2B1 PE=1 SV=2	sp|P22626	37 kDa	16	23
Heat shock protein HSP 90-alpha OS=Homo sapiens OX=9606 GN=HSP90AA1 PE=1 SV=5	sp|P07900	85 kDa	23	17
Actin, cytoplasmic 2 OS=Homo sapiens OX=9606 GN=ACTG1 PE=1 SV=1	sp|P63261	42 kDa	12	25
Tubulin alpha-1C chain OS=Homo sapiens OX=9606 GN=TUBA1C PE=1 SV=1	sp|Q9BQE3	50 kDa	0	57

Ubiquitin remnant pulldown and detection by LC-MS/MS. Expi293 cells treated with the proteasome inhibitor MG132 were lysed, and trypsin digested followed by SPE and lyophilization. The peptides were applied to the ubiquitin remnant immunoaffinity sorbent and eluted. The resulting peptides were analyzed by LC-MS/MS by HPLC using trapping and analytical columns with C18 resin and MS and MS/MS using a ThermoFisher™ Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer. Analysis was done using Scaffold Software™. Data shown are the top 11 detected proteins (out of a total of 2,252) from MG132-treated cells and untreated control cells.

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Laboratory Procedures

The Ubiquitin Remnant (K-ε-GG) Immunoaffinity Sorbent is designed for the immunoprecipitation of diglycyl-lysine containing peptide/protein ubiquitination remnants from biological samples. The immunoprecipitated material can be eluted and analyzed by LC-MS/MS proteomic analysis. Cells should be lysed and quantified, followed by trypsin digest prior to immunoprecipitation.

A typical procedure for immunoprecipitation is provided as follows:

1. Resuspend trypsin digested peptides in 1 ml cold IAP buffer.
2. Resuspend the sorbent by gentle flicking or pipetting (*do not vortex the sorbent at any point*).
3. Wash 40-100 µl (20-50 µl of bead volume) of the Ubiquitin Remnant (K-ε-GG) Immunoaffinity Sorbent using 1 ml of cold IAP buffer. Spin down at 500 x g for five minutes and discard the supernatant.
4. Repeat step (3) two additional times.
5. Add 40-100 µl of Ubiquitin Remnant (K-ε-GG) Immunoaffinity Sorbent (20-50 µl bead volume) to resuspended peptides.
6. Incubate the sorbent containing sample for one hour at 4° with inversion.
7. Centrifuge the beads at 500 x g for five minutes. Collect and save the supernatant for depletion analysis if desired.
8. Wash beads with 1 ml of cold IAP buffer and spin down at 500 x g for five minutes discarding the supernatant.
9. Repeat step (8) two additional times.
10. Wash beads with 1 ml cold PBS and spin down at 500 x g for five minutes discarding the supernatant.
11. Repeat step (10) two additional times.
    LC/MS analysis: Complete three additional washes with water to remove buffer salts/detergent. Resuspend the beads in 0.1% TFA in water, gently flick the tube and incubate for 5 minutes at room temperature. Separate the supernatant from the beads by centrifugation and the eluted sample is now ready for LC/MS analysis.

Description

Ubiquitin remnant (K-ε-GG) is a diglycyl-lysine motif found in proteins subject to ubiquitination, a process for tagging proteins for proteasomal degradation that is disrupted in certain diseases.[1,2] During ubiquitination, ubiquitin is covalently bound to the ε-NH_2 in lysine residues of the target protein *via* the C-terminal carboxyl group of ubiquitin, which is preceded by arginine-glycine-glycine (-RGG) residues.[3] Ubiquitinated proteins can be detected, and enriched for, *via* antibodies toward the K-ε-GG motif resulting from this covalent bond.[1,4] Cayman's Ubiquitin Remnant (K-ε-GG) Immunoaffinity Sorbent is designed for immunoprecipitation (IP) of ubiquitin remnant (K-ε-GG) motif-containing proteins from biological samples. This is an effective way to concentrate ubiquitin remnant (K-ε-GG) motif-containing proteins for subsequent detection and analysis by LC-MS/MS. The ubiquitin remnant (K-ε-GG) affinity sorbent consists of Cayman's Diglycyl-Lysine Monoclonal Antibody (Clone GX41) (Item No. 21096) coupled to CNBr-activated Sepharose 4B.

References

1. Walton, A., Stes, E., Cybulski, N., *et al.* It's time for some "site"-seeing: Novel tools to monitor the ubiquitin landscape in *Arabidopsis thaliana*. *Plant Cell* **28(1)**, 6-16 (2016).
2. Popovic, D., Vucic, D., and Dikic, I. Ubiquitination in disease pathogenesis and treatment. *Nat. Med.* **20(11)**, 1242-1253 (2014).
3. Hochstrasser, M. Evolution and function of ubiquitin-like protein-conjugation systems. *Nat. Cell Biol.* **2(8)**, E153-E157 (2000).
4. Udeshi, N.D., Svinkina, T., Mertins, P., *et al.* Refined preparation and use of anti-diglycine remnant (K-ε-GG) antibody enables routine quantification of 10,000s of ubiquitination sites in single proteomics experiments. *Mol. Cell. Proteomics* **12(3)**, 825-831 (2013).

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