V 1.1

Ubiquitin Affinity Control Beads

Cat. # CUB02B-Beads
Lot # 133
Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	1 x 220 µl when reconstituted
Validated applications:	IP
Species reactivity:	All

Background Information

Ubiquitin (Ub) and ubiquitin-like proteins (Ubls, e.g. SUMO, Nedd) are a group of approximately 15 proteins that have a molecular weight of around 8 kD. During the ubiquitination process, these are conjugated via activating (E1), conjugating (E2) and ligating (E3) enzymes to lysines of a target protein (1). Mammalian cells express over 600 potential ubiquitin ligases which exceeds that of the kinase superfamily of PTM proteins (2).

One function of ubiquitination is to target proteins for proteosomal degradation. This role can range from a general housekeeping function that clears miss-folded proteins from a cell to involvement in tightly regulated spatio-temporal cell signaling events (1). An emerging function of ubiquitination is its ability to activate proteins via the creation of unique protein:protein interactions (3). In common with many other PTMs, ubiquitination is reversible. Ubiquitin-specific proteases (USPs or DUBs) remove ubiquitins from target proteins (4). The reversible nature of ubiquitination further enhances the potential of this PTM to dynamically regulate protein function.

When a protein of interest (POI) is ubiquitinated the percentage of modified protein is very low i.e. usually <1% of the POI. This low level of modified protein is sufficient to mediate profound regulatory changes at the cellular level but it presents the technical challenge of detection. To detect the low level of modified protein requires some form of enrichment. Cytoskeleton's ubiquitin affinity beads are a powerful tool for the complete analysis of a POI ubiquitin profile (see Figure 1). When performing ubiquitinated protein enrichment it is important to include a control bead that is as similar to the enrichment beads as possible but lacks the ability to bind ubiquitinated proteins. The CUB02B-beads contain point mutations in the ubiquitin affinity binding domains of the ubiquitin enrichment beads (UBA01B-beads) and offer an excellent control bead for enrichment IPs.

Material

Ubiquitin affinity control beads contain crosslinked mutated Ubiquitin Binding Domains (UBDs/TUBES). The control beads are a powerful control for the complete analysis of a protein ubiquitin profile and allow the assessment of non-specific binding.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized CUB02B-Beads can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the lyophilized beads at the bottom of the tube. Reconstitute each tube in 220ul of Milli-Q water to achieve 50% slurry and store at 4°C. Allow the beads to rehydrate completely before use (2-5 minutes). Final buffer composition is 50 mM Tris pH 8.0, 5 mM EDTA, 5% sucrose, and 1% dextran. When stored and reconstituted as described, the product is stable for at least 6 months in 4°C.

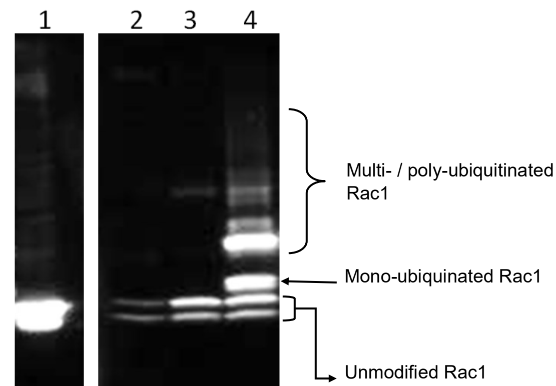
The part # has been updated from CUB02 to CUB02B beads due to a reformulation of bead buffer. Reformulation results in an enhanced bead performance post lyophilization. The affinity protein formulation on the beads has not changed.

Applications

Control bead to assess non-specific binding in immunoprecipitation (IP) Applications

Use 20 µl bead slurry for IP. Sufficient for 10 ubiquitin IP control reactions. See Figure 1 for representative data.

Figure 1: Detection of Endogenous Ubiquitinated Rac1



Swiss 3T3 cells were either untreated (Lane 3) or treated (Lanes 2 & 4) with bacterial toxin CNF1 for 3 hours prior to lysis in BLASTR™ buffer. Lysates (300 µg per assay) were treated as outlined in the Signal-Seeker™ Ubiquitin Enrichment manual, each reaction used 20 µl of beads. The western blot was probed with an anti-Rac1 antibody. CNF1 treatement resulted in detection of mono– and polyubiquitinated species of Rac1 which agrees with previous reports (5). Lane 1, 3% of input signal; Lane 2, control beads plus treated lysate; Lane 3, UBA01 beads plus untreated lysate; Lane 4, UBA01 beads plus treated lysate. The protease inhibitor MG132 was included in all lysates (10 µM/3h). Clear ubiquitin signals were also detected in the absence of MG132 (data not shown).

Application 1: Detection of Endogenously Ubiquitinated Rac1

The data shown in figure 1 agrees with published transfection data and demonstrates the utility of UBA01-Beads in studying the rapidly growing area of small G-protein regulation by ubiquitination (5-8). Control beads allow determination of specific Ub-Rac1 signal (Fig. 1 lane 2).

IP and WB Method:

1. Flick tube containing Ubiquitin Affinity Bead 1 suspension several times to make sure that the beads are completely resuspended in the tube.
2. For each IP assay, aliquot 20 µl of bead suspension into a tube on ice.
2. Flick tube containing Ubiquitin IP Control Bead suspension several times to make sure that the beads are completely resuspended in the tube.
3. Aliquot 20 µl of bead suspension for a control reaction to determine non-specific binding of ubiquitinated species.
4. Add lysate. We recommend 0.5-1.0 mg of lysate per assay as a starting point. NOTE: the amount of lysate required will vary depending upon the abundance of modified target protein. We also recommend that the IP reaction be carried out in a 50% RIPA buffer.
5. Save a small amount of lysate (20 µl) to run as a western input lysate control.
6. Incubate the tubes on a rotating platform at 4°C for 2h.
7. Collect beads by centrifugation at 3-5,000 x g for 1 minute at 4°C.
8. Aspirate off as much supernatant as possible without disturbing the beads.
9. Wash beads in Wash buffer such as 50% RIPA buffer for 5 minutes on a 4°C rotating platform.

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10. Collect beads by centrifugation at 3-5,000 x g for 1 minute at 4°C.
11. Aspirate off as much supernatant as possible without disturbing the beads.
12. Repeat the wash step two more times.
13. After the final wash, completely remove buffer supernatant without disturbing the bead pellet. Optional Technical Tip: remove residual supernatant using a fine bore protein loading tip.
14. Resuspend the beads in 30 µl of **2x non-reducing** SDS sample buffer (125mM Tris pH6.8, 20% glycerol, 4% SDS, 0.005% Bromophenol blue).
15. Mix the beads by gently tapping the end of the tube, we do not recommend using a pipette for this. Incubate the solution at room temperature for 5 min. Collect supernatant by centrifuge tube at maximum speed for 1 min at 4°C.
16. Add 1 ul of beta mercaptoethanol to a new Eppendorf tube. Carefully remove sample from step 6 without disturbing the beads and transfer to the new tube containing beta mercaptoethanol. Boil sample for 5 min prior to loading on SDS-PAGE.
17. Run protein sample in SDS-PAGE and perform western blot analysis on the POI.

Product Citations/Related Products

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