V 5.0

Ubiquitin Affinity Beads 1

Cat. # UBA01B-Beads
Lot:
Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	4 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 **Figures 1** & **2**).

Material

Ubiquitin affinity beads contain crosslinked Ubiquitin Binding Domains (UBDs), also called Ubiquitin-associated Domains (UBAs) and, in cases where multiple domains are expressed in a single protein, Tandem Ubiquitin Binding Entities (TUBES). Cytoskeleton scientists have developed a proprietary formulation of UBDs that have the unique characteristic of capturing both monoubiquitinated and polyubiquitinated proteins with high affinity. As mono-, multi– and poly-ubiquitination often confer unique, non-redundant properties to their target protein it is crucial to obtain the complete ubiquitin profile of any given target protein. Cytoskeleton's ubiquitin affinity beads are a powerful tool for the complete analysis of a protein ubiquitin profile.

As a control, affinity beads (CUB02B-Beads) that do not contain the ubiquitin affinity reagent have been included in this product. These allow the assessment of non-specific binding.

Storage and Reconstitution

Shipped at ambient temperature. The lyophilized UBA01B-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 220 µl of Milli-Q water to achieve 50% slurry and store at 4°C. Allow the beads to rehydrate completely before use (20-30 minutes). Final buffer composition is 80 mM PIPES pH 7.2, 4% sucrose, and 4% polyethylene glycol. When stored and reconstituted as described, the product is stable for at least 6 months in 4°C.

Reconstitute the control beads in 220 µl of Milli-Q water to achieve a 50% slurry and store at 4°C. Final buffer composition is 80 mM PIPES pH 7.2, 4% sucrose, and 4% polyethylene glycol. When stored and reconstituted as described, the product is stable for at least 6 months in 4°C.

The part #s have been updated from UBA01 and CUB02 to UBA01B and 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

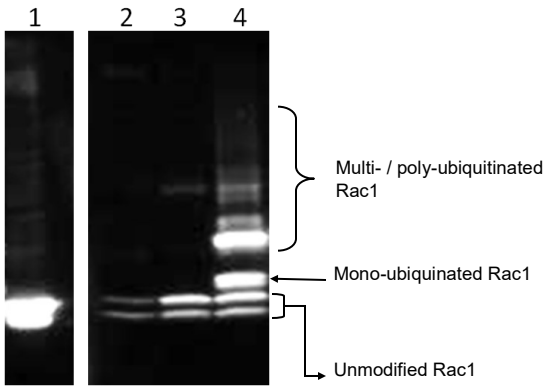
Immunoprecipitation (IP) Application

Use 20 µl bead slurry for IP. Sufficient for 40 ubiquitin IP reactions, there are sufficient control beads for 10 reactions. See **Figures 1** & **2** for representative data.

Application 1: Detection of Endogenously Ubiquitinated Rac1

The data shown in **Figure 1** agrees with published transfection data and demonstrates the utility of UBA01B-Beads in studying the rapidly growing area of small G-protein regulation by ubiquitination (5-8).

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).

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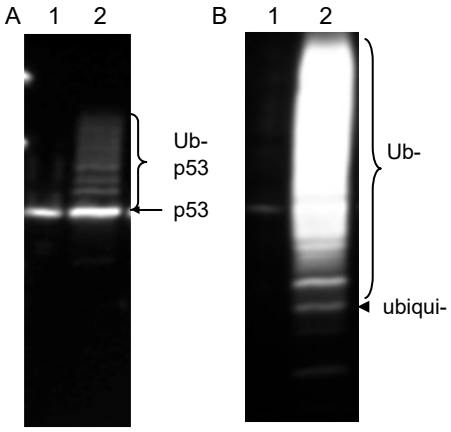
Application 2: Detection of Endogenously Ubiquitinated p53

Ubiquitination is known to be a major regulator of the tumor suppressor protein p53 (9). The data shown in **Figure 2** agrees with published data (9) and demonstrates the utility of UBA01-Beads.

Figure 2: Detection of Endogenous Ubiquitinated p53 and Total Ubiquitinated Proteins



HeLa cells were grown to 70% confluency and harvested by lysis in BLASTR™ buffer. Lysates (500 µg per assay) were treated as outlined in the Signal-Seeker™ Ubiquitin Enrichment manual, each IP reaction utilized 20 µl of UBA01 affinity beads. The western blot (A) was probed with an anti-p53 antibody and re-probed (B) with anti-ubiquitin-HRP antibody. **Lane A1**, 2% input lysate; **Lane A2**, IP from 500 µl of HeLa lysate enriched using ubiquitin affinity beads. Ubiquitinated p53 is clearly visible in the IP lane which agrees with published data (9). Western blot (B) shows blot (A) re-probed with anti-ubiquitin-HRP antibody. **Lane 1** shows a slight signal at the position of p53 as the blot was not stripped prior to re-probing.

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.
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

For the latest citations and related products please visit www.cytoskeleton.com

References

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