V 2.0

Mouse IgG IP Control Beads

Cat. # CIG01-Beads
Lot:
Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	1 x 330 µl when reconstituted
Validated applications:	IP control for Ab affinity reagents
Species reactivity:	na
Host/Isotype:	Mouse/polyclonal
Clone:	na

Background Information
Many of Cytoskeleton Inc's Signal-Seeker™ affinity enrichment beads are based on mouse monoclonal antibody reagents co-valently bound to beads. Mouse IgG IP Control Beads provide an ideal negative control and should be included in an IP experiment to control for non-specific binding in any antibody based affinity immunoprecipitation reaction, see **Figure 1**.

Material
Normal whole mouse IgG from non-immunized animals has been co-valently linked to agarose affinity beads. Antibody binding is in the range of 0.3-0.8 mg antibody per ml of bead slurry which is a similar range to Signal-Seeker™ affinity reagents.

Storage and Reconstitution
Shipped at ambient temperature. The lyophilized IP control 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 330ul of Milli-Q water to achieve 50% slurry and store at 4°C. Alternatively, reconstitute in 330 µl of 50% glycerol and store in –20°C. In both cases, allow beads to rehydrate completely before use (15-20 minutes). Final buffer composition is 200 mM PIPES, 5% sucrose, and 1% dextran. When stored and reconstituted as described, the product is stable for at least 6 months in 4°C and 12 months in –20°C.

Applications
Immunoprecipitation (IP) Application
Use an equivalent volume of control bead slurry as that being used for an enrichment IP assay. This is generally in the region of 30-40 µl per IP. Sufficient for 8-10 IP reactions. See **Figure 1** for representative data.

Figure 1: Enrichment of SUMOylated proteins from cell lysates

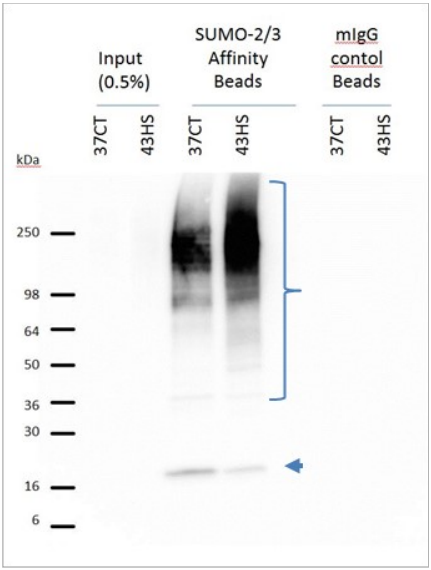

Figure Legend: A431 cell lysates were prepared from HS43: Heat Shock treated (43°C for 10min) and CT37: untreated cells. SUMO-2/3 conjugates were enriched from 1mg of lysate and immuno-blotted by anti-SUMO-2/3 antibody (Cytoskeleton Cat # ASM23) along with mouse IgG (mIgG) control beads (Cat # CIG01-Beads).

The level of total SUMO-2/3 conjugates in heat shock treated cell SUMO 2/3 Affinity Beads (Lane 43HS) is stronger than control cells (SUMO 2/3 Affinity Beads Lane 37CT). Total SUMOylated protein signal is delineated by the blue bracket, the blue arrowhead indicates the position of free SUMO 2/3. Lack of signal in the mouse IgG (mIgG) lanes demonstrates the specificity of the SUMO-2/3 bead reagent.

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