COSMO BIO CO., LTD.

Ubiquitin Monobody IP Beads & Buffer Kit

For research use only

Cat. No. CSR-MB-001-SAB

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Note: This product requires the separately sold Anti-Pan Ubiquitin Monobody, Biotin (Cat. No. MB-001-B) for use.

【 I 】Introduction

This product is a dedicated support kit designed to simplify and enhance the efficiency of immunoprecipitation (IP) using Anti-Pan Ubiquitin Monobody, Biotin (Cat. No. MB-001-B). It includes streptavidin magnetic beads, along with a set of buffers optimized for each step of the workflow, including lysate preparation, monobody immobilization, capture of ubiquitinated proteins, washing, and elution.

The Anti-Pan Ubiquitin Monobody, Biotin binds to ubiquitinated proteins regardless of ubiquitin chain length or linkage type, making it well suited for a range of downstream applications, including Western blot and proteomic analyses. Furthermore, because the amino acid sequence of ubiquitin is highly conserved across species, immunoprecipitation using Anti-Pan Ubiquitin Monobody, Biotin is applicable to a wide range of organisms.

A notable feature of this kit is its use of FG beads®—streptavidin magnetic beads coated with poly(glycidyl methacrylate). As a result, nonspecific adsorption, which is often problematic with conventional beads, is minimized, enabling the high-purity, low-background recovery of ubiquitinated proteins.

【 II 】Kit Components

Storage temperature: 4 °C
Number of assays: 20 (based on the usage described in the protocol)
Handling precautions: When handling this product, wear appropriate personal protective equipment such as safety glasses and gloves, and avoid direct contact with skin or eyes.

Contents	Vol	Qty	Remarks
Streptavidin Magnetic Beads	50 µL	1 vial	FG beads® (Tamagawa Seiki Co., Ltd., Cat. No. TAS8848N1170)
Monobody Immobilization Buffer (1x)	1.5 mL	1 vial	
Lysis/Wash Buffer (2x, pH 7.5)	30 mL	1 vial	This buffer contains 1 mM EDTA, 2% IGEPAL® CA-630, and 20% glycerol.
SDS Elution Buffer (4x, pH 6.8)	0.5 mL	1 vial	This buffer has a general composition suitable for SDS-PAGE with Tris-Glycine gels.
LDS Elution Buffer (4x, pH 8.5)	0.5 mL	1 vial	This buffer has a general composition suitable for SDS-PAGE with Bis-Tris gels.

【 III 】Additional Materials Required

- Anti-Pan Ubiquitin Monobody, Biotin (e.g., Cosmo Bio Co., Ltd. MB-001-B)
- Cell or tissue samples
- Magnetic Stand
- Rotary mixer (end-over-end type)
- Vortex mixer
- Ultrasonic disruptor (e.g., Bioruptor, Cosmo Bio Co., Ltd., UCD-250HSA or equivalent)
- Microcentrifuge (for ≥12,000 × g and spin-down steps) and 1.5 mL microcentrifuge tubes
- PBS (phosphate-buffered saline)
- Protease inhibitor cocktail
- Reducing agents (e.g., 2-mercaptoethanol, DTT)

【 IV 】Buffer Preparation

1. Monobody Immobilization Buffer

The supplied Monobody Immobilization Buffer (1x) is ready to use as is.

2. Lysis Buffer

Immediately before use, prepare a 1x Lysis Buffer by adding purified water and an appropriate protease inhibitor cocktail to the supplied Lysis/Wash Buffer (2x).

Select a protease inhibitor cocktail appropriate for your sample type.

In addition to general protease inhibitors, the following additives may also be effective:

- MG-132 (proteasome inhibitor; final concentration: 10 µM)
- PR-619 (deubiquitinating enzyme [DUB] inhibitor; final concentration: 10 µM)
- Iodoacetamide (DUB inhibitor; final concentration: 10 mM)

***Note**: These inhibitors are not included in this kit.*

3. Wash Buffer

Prepare a 1x Wash Buffer by diluting the supplied Lysis/Wash Buffer (2x) with purified water.

4. Elution Buffer

Select an appropriate elution buffer according to the intended downstream application.

If performing SDS-PAGE, prepare the elution buffer as follows:

- **When using Tris-Glycine gels:**
  Immediately before use, prepare a 1x elution buffer by adding purified water and a reducing agent (e.g., 0.1 M DTT final concentration) to the supplied ***SDS Elution Buffer (4x, pH 6.8)***.
- **When using Bis-Tris gels:**
  Immediately before use, prepare a 1x elution buffer by adding purified water and a reducing agent (e.g., 0.1 M DTT final concentration) to the supplied ***LDS Elution Buffer (4x, pH 8.5)***.

***Note:** Anti-Pan Ubiquitin Monobody exhibits high affinity for ubiquitin (Kd = 0.88 nM), and the Monobody-immobilized beads (see below) bind polyubiquitin chains in a multivalent manner. As a result, acidic bufferssuch as Glycine-HCl or TFA may not be effective for eluting ubiquitinated proteins. If acidic conditions are to beused, please verify their suitability in advance.*

【 V 】Sample Preparation

Using the lysis buffer included in this kit, lysates can be prepared from cells or tissues of various species. For adherent mammalian cells, please refer to the procedure described below. For other biological samples, consult relevant literature to determine an appropriate preparation method.

As a general guideline, 50 µL of cleared lysate at a concentration of 1 mg/mL is used per assay.

※ For samples that contain interfering substances—such as green plant tissues—perform cleanup (e.g., gel filtration) as needed before measuring protein concentration.

Preparation of Clear Lysate from Adherent Mammalian Cells (e.g., HCT116)

① Prepare cells cultured to a sub-confluent state in a 10 cm dish.
Note: *A total protein yield of approximately 1–2.5 mg is typically obtained from one 10 cm dish.*

② Remove the culture medium and wash the cells twice with 10 mL of PBS.

③ Add 1 mL of PBS, gently detach the cells using a cell scraper, and transfer the suspension to a 1.5 mL tube.

④ Centrifuge at 200 × g for 3 min at 4 °C.
Resuspend the pellet in 0.5 mL of PBS, centrifuge again under the same conditions, and discard the supernatant.

⑤ Add 200 µL of lysis buffer and resuspend thoroughly. Fragment the genomic DNA by sonication (e.g., 350–380 W, 10 s ON / 30 s OFF, 20 cycles).

⑥ Incubate the lysate on ice for 30 min, then centrifuge at ≥12,000 × g for 10 min at 4 °C.
Collect the supernatant as the clear lysate.

⑦ Measure the protein concentration and dilute with lysis buffer if necessary.
Note: *Prepared clear lysates can typically be stored at –70 °C or below for several months. However, we recommend confirming storage stability in advance depending on the sample type and intended application.*

【 VI 】 Preparation of Monobody-Immobilized Beads

Caution: When working with small liquid volumes, bead loss and pipetting errors are more likely to occur. To ensure consistent results, **always prepare Monobody-immobilized beads in batches for 10 assays.**

Reagent volumes per 10 assays:

- Anti-Pan Ubiquitin Monobody, Biotin (sold separately): 30 µL (equivalent to 15 µg)
- Streptavidin Magnetic Beads (included): 25 µL (equivalent to 0.5 mg)

Note: *At this loading, the monobody is nearly saturated on the beads.*

① Vortex the streptavidin magnetic beads at medium speed for at least 30 seconds to ensure complete resuspension.

② Transfer 25 µL of the bead suspension to a 1.5 mL tube.

③ Place the tube on a magnetic stand and let the beads fully separate. Then, carefully remove the supernatant, avoiding disturbance of the beads.
Note: *FG beads are very small (0.2 µm diameter) and highly dispersible, so separation may take several minutes.*

④ Add 200 µL of Monobody Immobilization Buffer to resuspend the beads, then perform a brief spin down and magnetic separation to remove the supernatant.

⑤ Add 70 µL of Monobody Immobilization Buffer to resuspend the beads.

⑥ Add 30 µL of Anti-Pan Ubiquitin Monobody, Biotin, and mix thoroughly by pipetting.

⑦ Incubate at 4℃ for 1 hour at a moderate rotation speed using an end-over-end rotator.
Note: *During incubation, occasionally check to ensure the beads are being adequately mixed, and adjust the rotation speed as needed. Temporary microaggregation may be observed after incubation, but it will naturally resolve during the immunoprecipitation reaction.*

⑧ After incubation, perform a brief spin down and magnetic separation, then discard the supernatant (flow-through). If the supernatant is needed for downstream analysis or other purposes, transfer it to a separate tube for storage.

⑨ Add 500 µL of Wash Buffer to resuspend the beads, then perform a brief spin down and magnetic separation to remove the supernatant.
Repeat this wash step a total of three times.

⑩ Finally, resuspend the beads in 200 µL of Wash Buffer.

⑪ Store at 4 °C until use.
Note: *The prepared monobody-immobilized beads are stable at 4 °C for at least one month, but we recommend using them as soon as possible.*

【 Ⅶ 】 Immunoprecipitation Protocol

As initial conditions, we recommend using 50 µL of clear lysate at 1 mg/mL per assay, followed by overnight incubation at 4 °C. These conditions are based on experiments using HCT116 cells and may be adjusted as needed depending on the sample type and experimental goals.

Please refer to section [III] Buffer Preparation and select an appropriate elution buffer according to your downstream application.

① Vortex the monobody-immobilized beads at medium speed for at least 30 seconds to ensure complete resuspension.

② Transfer 20 µL (0.05 mg) of the bead suspension to a 1.5 mL microcentrifuge tube.

③ Place the tube on a magnetic stand to collect the beads, and carefully remove the supernatant.

④ Add 50–100 µL of clear lysate and gently resuspend the beads by pipetting.

⑤ Incubate overnight at 4 °C with gentle rotation using an end-over-end rotator.

⑥ After incubation, perform a brief spin down and magnetic separation, then discard the supernatant (flow-through). If needed, transfer the supernatant to a separate tube for retention.

⑦ Add 200 µL of Wash Buffer, vortex to resuspend, then perform a spin down and magnetic separation to remove the supernatant.
Repeat this wash step a total of three times. On the third wash, to prevent nonspecific proteins adsorbed on the inner wall from being carried over into the eluate, transfer the suspension to a new tube before magnetic separation.

⑧-A **If using Tris-Glycine gels:** Add 20–50 µL of **SDS Elution Buffer**, gently resuspend the beads by pipetting, and heat at 95 °C for 5 minutes.

⑧-B **If using Bis-Tris gels:** Add 20–50 µL of **LDS Elution Buffer**, gently resuspend the beads by pipetting, and heat at 70 °C for 10 minutes.
Note: *The Anti-Pan Ubiquitin Monobody exhibits high affinity for ubiquitin (Kd = 0.88 nM), and the monobody-immobilized beads bind polyubiquitin chains multivalently.*
Heat treatment is therefore essential for complete elution.

⑨ After spin-down, perform magnetic separation and transfer the supernatant (eluate) to a new tube. If necessary, centrifugation (e.g., ≥ 12,000 × g for 1 min) can be used as an alternative to magnetic separation.

⑩ Use the eluted sample immediately or store at –20 °C for short-term storage. For long-term storage, –70 °C or lower is recommended.

【 Ⅶ 】Example Application: Immunoprecipitation–Western Blotting (IP–WB)

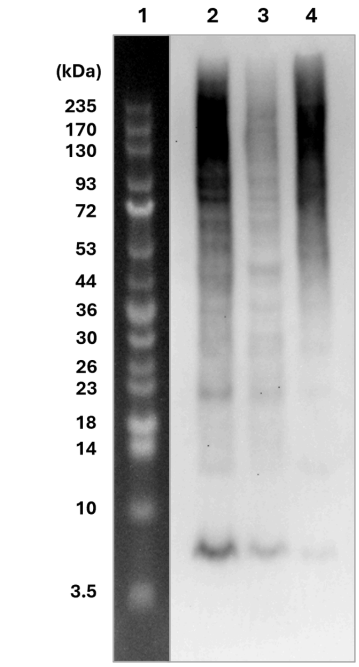

Figure 1. Immunoprecipitation Using Mammalian Cells

Lysate was prepared from untreated human HCT116 cells according to the protocol described in this datasheet. Immunoprecipitation was performed using monobody-immobilized beads with 50 µL of lysate. Ubiquitinated proteins bound to the beads were eluted with 50 µL of 1× LDS Elution Buffer. To evaluate the recovery efficiency of ubiquitinated proteins, the eluted fraction was diluted to match the volume of the lysate and flow-through fractions, and each sample was subjected to SDS-PAGE using a 4–12% Bis-Tris gel. The separated proteins were transferred onto a PVDF membrane and blocked with 3% skim milk. Ubiquitinated proteins were detected using a mouse monoclonal anti-ubiquitin primary antibody (P4D1, 1:1000 dilution) and an HRP-conjugated goat polyclonal anti-mouse IgG secondary antibody (1:2000 dilution), followed by ECL-based chemiluminescence detection.

Lane 1: Molecular weight marker
Lane 2: Lysate
Lane 3: Flow-through
Lane 4: Eluate

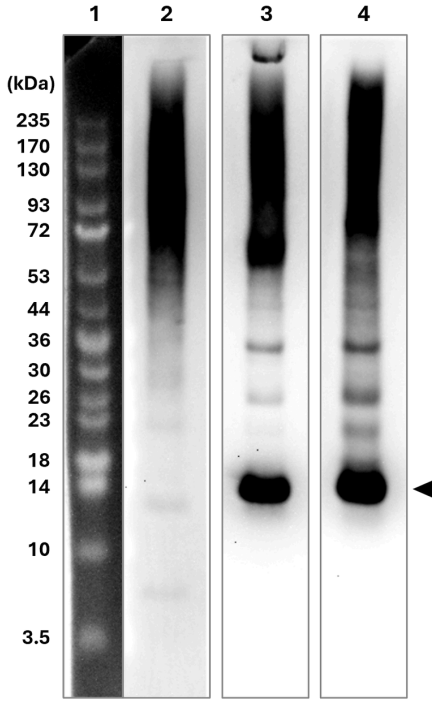

Figure 2. Comparison of Signal Patterns by Detection Method

Using eluted fractions obtained from the same immunoprecipitation assay as described in Figure 1, three PVDF blots were prepared, each employing a different combination of blocking reagent, primary antibody, and secondary reagent (see lane descriptions below). Ubiquitinated proteins were detected on each blot using the ECL method. Notably, in lanes 3 and 4, in addition to the ubiquitinated proteins, the Anti-Pan Ubiquitin Monobody, Biotin dissociated from the streptavidin beads was also detected (arrowhead), highlighting differences in signal patterns depending on the detection system.

Lane 1: Molecular weight marker
Lane 2: 3% skim milk / anti-ubiquitin mouse monoclonal antibody (P4D1, 1:1000 dilution) / HRP conjugated goat anti-mouse IgG polyclonal antibody (1:2000 dilution)
Lane 3: 3% BSA / Anti-Pan Ubiquitin Monobody, Biotin (1:5000 dilution) / HRP-conjugated anti-6xHis mouse monoclonal antibody (1:2000 dilution)
Lane 4: 3% BSA / Anti-Pan Ubiquitin Monobody, Biotin (1:5000 dilution) / HRP-conjugated streptavidin (1:5000 dilution)

【 Ⅶ 】Example Application: IP–WB (continued)

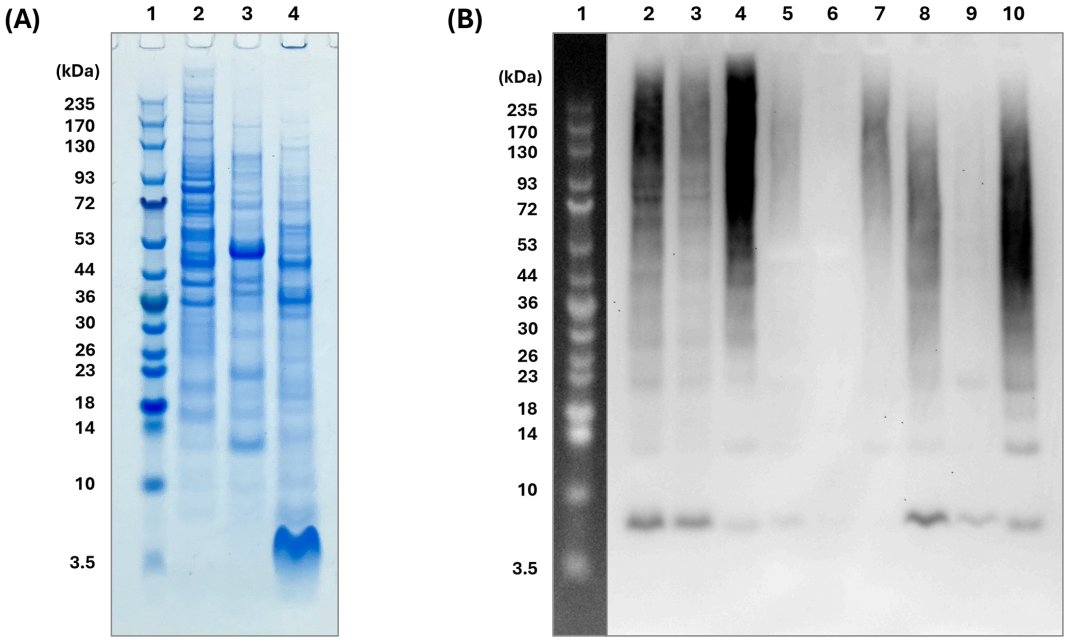

Figure 3. Broad Applicability Across Species

(A) Lysates were prepared using the lysis buffer included in this kit from human HCT116 cells (**Lane 2**), *Arabidopsis* leaves (**Lane 3**), and *Rhizopus* hyphae (**Lane 4**). For *Arabidopsis* and *Rhizopus*, lysates were pretreated by gel filtration prior to protein quantification. Each lysate was adjusted to 1 mg/mL, and 5 µg of protein was separated by SDS-PAGE followed by CBB staining. This allowed visual confirmation that the protein concentrations were approximately equivalent across the samples.

Lane 1: Molecular weight marker

(B) Immunoprecipitation, SDS-PAGE, and western blotting were performed following the same protocol as in Figure 1, except that no dilution was applied to the eluted fractions.

Lane 1: Molecular weight marker
Lanes 2–4: Lysate, flow-through, and eluate from human HCT116 cells
Lanes 5–7: Lysate, flow-through, and eluate from *Arabidopsis* leaves
Lanes 8–10: Lysate, flow-through, and eluate from *Rhizopus* hyphae

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