HA-Tag IP/Co-IP Kit

A1455938

Storage Temperature 4, −20°C (For detailed storage conditions, please refer to the Kit Components).

Shipping Conditions Shipped with ice packs. Upon receipt, please promptly store the product under the recommended storage conditions.

Product Introduction

Anti-HA Affinity Chromatography Medium is based on a 4% agarose gel matrix, which exhibits low non-specific binding to miscellaneous proteins. This kit contains high-quality Anti-HA agarose gel and optimized, validated essential reagents for immunoprecipitation, making procedures such as immunoprecipitation (IP, also referred to as Pull-down) and co-immunoprecipitation (Co-IP) simpler, more convenient, and highly efficient. It is widely applicable for the immunoprecipitation, co-immunoprecipitation, or purification of HA-tagged fusion proteins and their associated protein complexes.

Tags like HA-tag, Myc-tag, His-tag, Flag-tag, V5-tag and GST-tag are among the most common tags found in expression vectors. The fusion expression of target proteins with these tags facilitates convenient detection of the target protein and proteins that interact with it, as well as simplifies the purification of the target protein. The storage temperature for components includes 4 °C and -20 °C; for detailed storage information, please refer to Table 1. Kit Components.

Table1. Kit Components

A1455938	Components	20T	100T	Storage Temperature	Quantity Per Test
A1455938A	Anti-HA resin	0.4 mL	2 mL	4°C	20 μL per 250 μg sample
A1455938B	1×Lysis Buffer	20 mL	100 mL	4°C	150 μL per 250 μg sample
A1455938C	HA Peptide	0.2 mL	1 mL	−20°C	10 μL per 100 μL wash Buffer
A1455938D	Acid Elution Buffer	2 mL	10 mL	4°C	100 μL per 250 μg sample
A1455938E	10×Wash Buffer	40 mL	200 mL	4°C	0.5 mL per 250 μg sample
A1455938F	Neutralization Buffer	0.2 mL	1 mL	4°C	10 μL per 100 μL Acid Elution Buffer
A1455938G	Protease Inhibitor Cocktail(100×)	0.2 mL	1 mL	−20°C	1.5 μL per 150 μL Lysis buffer
A1455938H	2×SDS−PAGE Loading buffer	0.4 mL	2 mL	−20°C	20 μL per 250 μg sample

Table 2. Performance of HA Aagarose Beads

Parameters	Specifications
Matrix	4% Agarose Beads
Ligand	Anti-HA Mouse Monoclonal Antibody
Particle Size Range	45–165 μm
Binding Capacity	>1 mg HA-Tagged Protein per mL of Medium
Bead Volume	Gel Volume Accounts for 20% of Suspension Volume
Storage	0.1% ProClin 300, 2–8°C

Procedure (Unless otherwise specified, all procedures should be performed at 4°C)

1 Reagent Preparation

Additional Required Materials:

(1) Reagents to be prepared by the user:

a) Primary Antibody: HA tag antibody.

b) Secondary Antibodies: Goat Anti-Mouse IgG H&L (HRP), Goat Anti-Rabbit IgG H&L (HRP).

c) Other Reagents: TBST, Electrophoresis Buffer, Transfer Buffer, Reducing SDS-PAGE Gel.

(2) Required Equipment:

Electrophoresis Apparatus, Transfer Apparatus, Imaging System.

The above reagents, if required, can be ordered from Aladdin: HA tag Mouse mAb (Ab106781)、Recombinant HA tag Antibody (Ab106771)、Goat Anti-Rabbit IgG H&L (HRP) (Ab176443)、Recombinant Protein A/G, HRP conjugate(rp303272)、Goat Anti-Mouse IgG H&L (HRP) (Ab179001).

2 Solution Preparation

You may use the buffers provided in the kit, or prepare different buffer systems according to actual needs. It is recommended to filter all buffers through a 0.22 μm or 0.45 μm membrane filter before use. Buffers should be stored at 4°C. If any reagent appears cloudy, discard it immediately.

(1) Prepare an appropriate amount of inhibitor-containing lysis buffer based on the proportion of using 100–200 μL of inhibitor-containing lysis buffer for lysis and 300–600 μL of inhibitor-containing lysis buffer for washing per 0.5–1 million cells. Mix Lysis Buffer and Protease Inhibitor Cocktail (100×) at a ratio of 100:1. For example, add 10 μL of Protease Inhibitor Cocktail (100×) to 1 ml of Lysis Buffer to obtain 1 ml of inhibitor-containing lysis buffer (Lysis Buffer with Protease Inhibitor Cocktail). The prepared inhibitor-containing lysis buffer should be placed on ice or at 4°C.

Note: The inhibitor-containing lysis buffer should be prepared fresh before use and should not be frozen and stored for later applications.

(2) Preparation of HA Peptide Elution Buffer (10 mg/mL): Dilute a certain amount of the HA peptide stock solution with wash buffer to a final concentration of 150 μg/mL. For instance,

add 10 μL of the peptide to 90 μL of wash buffer and mix well to achieve a final concentration of 150 μg/mL. Adjustments can be made according to experimental requirements.

(3) Preparation of 10×Wash Buffer: Dilute the 10×wash buffer with deionized water at a 9:1 ratio. For example, add 9 mL of deionized water to 1 mL of 10×wash buffer, and mix well to obtain the 1×wash buffer.

(4) Resin Washing: Gently resuspend the Anti-HA Resin to form a homogeneous gel suspension. Typically, use 20 μL of the well-mixed gel suspension per 250 μg (the following immunoprecipitation steps are described based on adding 20 μl of gel suspension per sample). Transfer an appropriate amount of Anti-HA Resin into a clean centrifuge tube, and add 1×wash buffer to a final volume of approximately 0.5 mL. Repeat the above steps twice.

Note: Using wide-bore tips (e.g., by cutting off the tip end with scissors) may facilitate pipetting of the gel suspension.

3 Preparation of Test Samples (**Note**: Perform all sample lysis steps at 4°C or on ice)

(1) For the preparation of serum samples:

If the target protein is abundant, dilute the serum sample with Lysis Buffer to a final target protein concentration of 50–150 μg/mL. Keep the diluted sample on ice for immediate use or store at -20°C for long-term preservation.

(2) For Adherent Cell Lysis and Preparation:

Aspirate the culture medium and wash the cells twice with PBS. Remove all residual liquid completely. Add 100-200 μL of Lysis Buffer with inhibitors per 0.5-1 million cells (equivalent to one well of a 6-well plate). Pipette gently to ensure thorough contact between the lysis buffer and cells. Animal cells are typically lysed within 1-2 seconds of contact with the buffer. For plant cells, lyse on ice for 2–10 minutes. After complete lysis, use a cell scraper to detach the cells and transfer the lysate to a 1.5 mL microcentrifuge tube. Centrifuge at 10,000–14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before proceeding to subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, may be present after lysis and will form a pellet upon centrifugation.

(3) For Suspension Cell Lysis and Preparation:

Collect cells by centrifugation at 250-1,000×g for 5 minutes at room temperature. Wash the pellet twice with PBS and remove all residual liquid completely. Gently vortex or tap the tube to disperse the cells. Add 100–200 μL of Lysis Buffer with inhibitors per 0.5–1 million cells. Mix and incubate on ice for 5–20 minutes (mix several times during incubation). Tap the tube or pipette gently to ensure complete cell lysis; no significant cell pellet should remain after thorough lysis. If processing numbers of cells, aliquot them into tubes containing 0.5-1 million cells per tube before lysis. Large cell clumps are more difficult to lyse completely, whereas smaller numbers of cells allow better contact with the lysis buffer and lyse more efficiently. After complete lysis, centrifuge at 10,000-14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immunoprecipitation or co-

immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, may be present after lysis and will form a pellet upon centrifugation.

(4) For Bacterial or Yeast Sample Lysis and Preparation:

For 1 mL of bacterial or yeast culture, centrifuge to pellet the cells and remove the supernatant. Wash the pellet twice with PBS and remove all residual liquid completely. Gently vortex or tap the tube to resuspend and disperse the bacterial or yeast cells. Add 100–200 µL of Lysis Buffer with inhibitors. Gently vortex or tap the tube to mix, and lyse on ice for 2–10 minutes. For improved lysis efficiency, bacteria and yeast can be pretreated with lysozyme and lyticase, respectively, before adding the Lysis Buffer with inhibitors. After complete lysis, centrifuge at 10,000-14,000×g for 3-5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, is likely present after lysis and will form a pellet upon centrifugation.

(5) For Tissue Sample Lysis and Preparation:

Mince the tissue into small fragments. Add approximately 100–200 µL of Lysis Buffer per 20 mg of tissue. Homogenize the mixture using a glass homogenizer or other suitable homogenization device. Thorough homogenization ensures complete tissue lysis. After lysis, centrifuge at 12,000×g for 5 minutes at 4°C. Collect the supernatant for protein concentration determination before subsequent immunoprecipitation or co-immunoprecipitation experiments.

Note: A small amount of insoluble material, primarily genomic DNA, is likely present after lysis and will form a pellet upon centrifugation.

4 Immunoprecipitation (IP)

(1) Add Resin and Incubate: Add the magnetic beads to the protein sample at a ratio of 20 µL bead suspension per 250 µg of protein sample. Place the tube on a rocking platform or rotator and incubate at room temperature for 2 hours or at 4°C overnight.

(2) Centrifugal separation: After incubation, Centrifuge at 2000 rpm for 2 minute at 4°C. Wait until all resin are captured, then transfer the supernatant to a new centrifuge tube. Retain this supernatant for potential future analysis.

(3) Washing: Add 0.5 mL of Wash Buffer containing inhibitors and gently resuspend the beads by pipetting. Centrifuge at 2000 rpm for 2 minute at 4°C, then remove and discard the supernatant. Repeat this wash step three times using Lysis Buffer with inhibitors. This yields the bait protein-resin complex.

Note: The completeness of washing can also be assessed by measuring the OD_{280} of the wash supernatant. If the OD_{280} is greater than 0.05, appropriately increase the number of washes.

5. Protein Elution

Depending on the characteristics of the tagged protein and the requirements of subsequent experiments, choose one of the following three methods for elution.

(1) Non-Denaturing Elution

Add 100 µL of HA Peptide Elution Buffer per 20 µL of original resin volume. Mix well and place the tube on a rocking platform or rotator. Incubate with shaking at room temperature for 30-60

minutes, or at 4°C for 1-2 hours. To improve elution efficiency, the incubation time can be extended or the elution step can be repeated. Gently mix 3-5 times during incubation. After incubation, Centrifuge at 2000 rpm for 2 minute at 4°C. Once the beads are fully captured, aspirate the supernatant into a new centrifuge tube for Western Blot analysis.

(2) Elution with SDS−PAGE Loading Buffer

For every 20 µL of original bead volume, add 30 µL of PBS (user to provide) to resuspend the beads, followed by 30 µL of 2×SDS Loading Buffer. Mix gently by flicking the tube, then heat at 95-100°C for 5-10 minutes. Centrifuge at 1000 rpm for 1 minute. Collect the supernatant for SDS−PAGE electrophoresis or Western Blot analysis.

Note: Elution with SDS-PAGE Loading Buffer enables complete detachment of the target protein from the beads, but the antibody will also be completely eluted. The Anti-HA antibody on the resin is mouse-derived; therefore, it is recommended to select a rabbit antibody for the subsequent WB experiment. The recommended loading volume for Western Blot detection is within 20 µL. The remaining sample can be stored frozen at -20°C.

(3) Acidic Elution

Add 100 µL of Acid Elution Buffer per 20 µL of original resin volume. Mix well and place the tube on a rocking platform or rotator. Incubate at room temperature for 5 minutes. Centrifuge at 1000 rpm for 1 minute at 4°C, then transfer the supernatant to a new centrifuge tube. If neutralization is required, add 10 µL of Neutralization Buffer to adjust the pH to neutral.

Note 1: Although efficient, acid elution may still be less effective than competitive elution or SDS-PAGE loading buffer elution.

Note 2: As differences in target proteins may influence the efficiency of acid elution, if high elution efficiency is required, the pH of the Acid Elution Buffer can be adjusted within the range of 2.5-3.0; correspondingly, the pH or volume of the Neutralization Buffer should also be adjusted. Specific experimental conditions need to be optimized by the user. Alternatively, consider using the potentially more efficient HA Peptide Competitive Elution method or the expected highest efficiency SDS-PAGE Loading Buffer Elution method. The disadvantage of the latter is that elution occurs under denaturing conditions, which may impact subsequent experiments requiring protein activity.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
No Bait Protein in Elution Fraction	Protein may be in inclusion bodies, not in the supernatant.	Check if the supernatant contains the target protein by analyzing the lysate via electrophoresis. Proteins in inclusion bodies require purification methods specific for inclusion bodies.
	Expression level is too low	Optimize expression conditions
	HA tag is expressed but inactive	Optimize expression conditions
Non-specific bands are prominent	Insufficient washing during western blot	Increase the number of wash steps
Target Protein Not Captured	Insufficient amount of target protein in the sample	Verify protein expression or lysis efficiency by SDS-PAGE or Western Blot. Increase the amount of target protein to the recommended level
	Protein-protein interaction is too weak or non-existent	Optimize the Lysis/Wash Buffer; Add cofactors required for the protein interaction.
	Protein degradation	Add protease inhibitors; For temperature-sensitive proteins, perform all operations at 4°C or on ice.

Important Notes

1. Agarose beads usage should be optimized based on experimental requirements.
2. Before performing protein-protein interaction operations, be sure to read this manual carefully.
3. Unless otherwise specified, all steps are recommended to be performed at 4 °C to minimize potential protein degradation. If cell lysis is insufficient, sonication may be used after adding the lysis buffer.
4. The resin should be stored in the storage solution and protected from drying. Mix thoroughly before use.
5. Agarose beads that have been boiled lose their binding capacity and should not be reused.
6. This product is intended for research use by qualified professionals only.
7. Please follow safety guidelines and adhere to laboratory reagent handling protocols.
8. The IP lysis buffer included in this kit already contains protease inhibitors. If special needs arise, other appropriate inhibitor cocktails may be considered.
9. HA Agarose beads must be fully resuspended before use, i.e., invert the tube several times to ensure thorough mixing.
10. After collection, protein samples should be purified as soon as possible and always kept at 4 °C or on ice to slow down protein degradation or denaturation.