MCE USA
Tel: 609-228-6898
Email: tech@MedChemExpress.com

Anti-HA Affinity Gel

1 Packing list

Components	HY-K0232-1 mL	HY-K0232-5 mL	HY-K0232-10 mL
Anti-HA Affinity Gel	1 mL	5 mL	10 mL

2 Introduction

The HA tag corresponds to the 98-106 amino acid sequence of human influenza haemagglutinin, denoted by the sequence YPYDVPDYA.
MCE Anti-HA Affinity Gel is produced by covalently coupling high-quality HA antibody with agarose. It features a high loading capacity, high specificity, and stable properties. The Anti-HA Affinity Gel can recognize C and N-terminal HA tagged fusion proteins, making it suitable for detecting and purifying HA fusion expressed proteins. It can also be used in immunoprecipitation (IP) experiments to detect the expression of recombinant proteins in target cells.
The product contains 0.5 mL of gel per 1 mL of total volume. Prior to use, ensure the gel is thoroughly resuspended and mixed before aspiration.

3 Characteristics

Information

Composition	4% cross-linked agarose
Ligand	Anti-HA Mouse Monoclonal Antibody
Binding Capacity	> 1 mg of HA tagged protein/mL of Resin
Bead Diameter	45-165 μm
Pressure	0.3 MPa, 3 bar
Storage Solution	1× PBS，0.02% NaN_3

4 General Protocol

Buffer Preparation

Binding/Washing Buffer	50 mM Tris, 0.15 M NaCl, pH 7.4
Elution Buffer I	0.1 M Glycine, pH 2.0-2.8
Elution Buffer II	50 mM Tris, 0.15 M NaCl, 100-500 μg/mL HA Peptide, pH 7.4
Neutralization buffer	1 M Tris-HCl, pH 8.0
Storage Buffer	PBS，0.02% (w/v) NaN_3

Note: It is recommended to prepare all buffers with ultrapure water and. After preparation, filter them through a 0.45 μm or 0.22 μm membrane for sterilization.

Protein purfication

It is recommended to filter the sample with a 0.22 μm or 0.45 μm filter before purification.

Medium-pressure chromatography

1. Load the column: Load Anti-HA Affinity Gel in an appropriate chromatography column and connect it to the chromatography system.
2. Equilibration: Equilibrate the column by washing it with 5× column volume of Binding Buffer. Repeat 2-3 times.
3. Loading: Loading sample using a pump or sample loops, collecting the effluent. Repeating this loading process can improve the binding efficiency.
   Note: a. Please choose a suitable gel volume according to the amount of protein to avoid exceeding the loading capacity of agarose.
   b. An increase in sample viscosity or volume may lead to column backpressure.
4. Washing: Wash the column with approximately 15-30× column volume of Washing Buffer to remove nonspecifically adsorbed hetero-proteins and collect the wash effluent until the absorbance at 280 nm stabilizes.
5. Elution
   Two recommended elution methods depend on protein characteristics or further usage.
   1) Acidic condition Elution with Elution Buffer : Elute the column with approximately 3-5× column volume of Elution Buffer I, collect the eluent and immediately neutralize its pH with Neutralization Buffer (1/10 volume of total eluent volume), the collected eluates contain the target protein.
   Note: The agarose should be balanced with Washing Buffer immediately after acidic elution, and the agarose should not be kept in the Elution Buffer for more than 20 min.
   2) Competitive Elution: Elute the column with approximately 3-5× column volume of Elution Buffer II, the collected eluates contain the target protein.
   Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.
6. Regeneration: Thoroughly elute the column with 5× column volume of Elution Buffer I or II. Equilibrate the column with Washing Buffer.
   Note: The agarose should be balanced with Washing Buffer immediately after acidic elution, and the agarose should not be kept in the Elution Buffer for more than 20 min.
7. Storage: Equilibrate the resin with 5-10× column volume of Storage Buffer, disconnected the column and store at 2-8°C.

Gravity column method

1. Load the column: Choose an appropriate volume of Anti-HA Affinity Gel suspension according to the amount of samples and load it into the gravity chromatography column, eliminating the protective solution.
2. Equilibration: Equilibrate the column: Equilibrate the resin with 5× column volume of Binding Buffer, repeat 2-3 times.
3. Loading: Loading sample and collect the effluent. It is recommended that the samples maintain contact with the agarose at least 2 min.
   Note: Repeated loading can improve the binding efficiency.
4. Washing: Wash resin with 10-15× column volume of Washing Buffer to eliminate non-specifically adsorbed hetero-proteins, collect the effluent.
5. Elution
   Two recommended elution methods depend on protein characteristics or further usage.
   1) Acidic condition Elution with Elution Buffer I : Elute the column with approximately 3-5× column volume of Elution Buffer I, collect the eluent and immediately neutralize its pH with Neutralization Buffer (1/10 volume of total eluent volume), the collected eluates contain the target protein.
   Note: The agarose should be balanced with Washing Buffer immediately after acidic elution, and the agarose should not be kept in the Elution Buffer for more than 20 min.
   2) Competitive Elution: Elute the column with approximately 3-5× column volume of Elution Buffer II, the collected eluates contain the target protein.
   Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.
6. Regeneration: Thoroughly elute the column with 5-10× column volume of Elution Buffer. Equilibrate the column with Washing Buffer.
   Note: The agarose should be balanced with Washing Buffer immediately after acidic elution and the agarose should not be kept in the Elution Buffer for more than 20 min.
7. Storage: Wash the column with 5× the volume of Storage Buffer and store at 2-8°C.

Centrifugation

1. Preparation of Agarose: Choose an appropriate volume of Anti-HA Affinity Gel suspension according to the amount of samples and transfer it into a tube, centrifuge at 5,000 g for 1 min and discard. Wash the resin with 5× the volume of Washing Buffer, centrifuge at 5,000 g for 1 min and discard the supernatant. Repeat 2-3 times.
2. Binding: Add the sample and incubate at 4°C for 2-4 h (or 37°C for 0.5-2 h). Centrifuge at 5,000 g for 1 min and discard the supernatant (e.g., if required, save supernatant for subsequent analysis).
3. Washing: Wash the resin with 5× the volume of Washing Buffer, centrifuge at 5,000 g for 1 min and discard the supernatant. Repeat 3-5 times.
4. Elution:

   Two recommended elution methods depend on protein characteristics or further usage.

   1) Acidic condition Elution with Elution Buffer I : Elute the column with approximately 3-5× column volume of Elution Buffer I, incubate at room temperature for 5-10 minutes, centrifuge at 5,000 g for 1 min. The collected supernatant contains the target protein. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), then the samples can be used for functional analysis. Repeat 2-3 times and collect the supernatant each time.

      Note: The agarose should be balanced with Washing Buffer immediately after acidic elution, and the agarose should not be kept in the Elution Buffer for more than 20 min.

   2) Competitive Elution: Elute the column with approximately 3-5× column volume of Elution Buffer II, incubate at room temperature for 5-10 min, centrifuge at 5,000 g for 1 min. The collected supernatant contains the target protein. Repeat 2-3 times and collect the supernatant each time.

      Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.
5. Regeneration and storage: Wash the resin with 5-10× the volume of Washing Buffer, then wash the resin with 5-10× the volume of ddH2O. Finally, wash the resin with 2× the volume of Storage Buffer and store at 2-8°C.

IP

1. Preparation of Agarose

   1) Add 40 μL of Anti-HA Affinity Gel suspension (approximately 20 μL of gel) to a 1.5 mL tube, centrifuge at 5,000 g for 1 min and discard the supernatant.

   2) Add 500 μL of Binding Buffer and mix well, centrifuge at 5,000 g for 1 min and discard the supernatant. Repeat 3-4 times.
2. Sample Binding

   1) Add 200-1,000 μL of sample and mix thoroughly, gently rotate the tube at 4°C for 2 h. If you want to enhance the binding efficiency, you can incubate it overnight.

      Note: For proteins prone to degradation, it is recommended to add protease inhibitors.

   2) Centrifuge at 5,000 g for 1 min transfer the supernatant to a new centrifuge tube (the supernatant can be used to detect any residual HA-tagged protein).
3. Washing

   Add 1 mL Washing Buffer to the agarose and mix thoroughly, centrifuge at 5,000 g for 1 min and discard the supernatant. Repeat 2-3 times until the OD_{280} of the supernatant liquid is < 0.05.
4. Elution

   Three recommended elution methods depend on protein characteristics or further usage.

   1) Acidic condition Elution with Elution Buffer I: This method maintains the original biological activity of the eluted samples and is suitable for subsequent functional analyses.

      Add 50-100 μL of Elution Buffer I to the tube. Mix well and incubate for 5-10 min at room temperature, centrifuge at 5,000 g for 1 min and transfer the supernatant to a new tube. The final collected eluate is acidic, Neutralization Buffer should be added immediately to adjust the pH (1/10 volume of total eluent volume), then the samples can be used for functional analysis.

      Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.

   2) Competitive Elution with Elution Buffer : This method maintains the original biological activity of the eluted samples and is suitable for subsequent functional analyses.

      Add 50-100 μL of Elution Buffer II to the tube. Mix well and incubate for 5-10 minutes at room temperature, centrifuge at 5,000 g for 1 min and transfer the supernatant to a new tube.

      Note: The eluates can be used immediately if stored at 4ºC, or stored at -20ºC for long term storage.

3) Gel Electrophoresis and Immunoblotting Elution with SDS-PAGE Loading Buffer.

Add 20-50 μL of 2× SDS-PAGE Loading Buffer to the tube. Mix well and heat for 5 min at 95ºC. Centrifuge at 5000 g for 1 min. Reserve the supernatant for SDS-PAGE analysis.

Note: As regular SDS-PAGE Loading Buffer contains β-mercaptoethanol and DTT, which can break the light and heavy chains of the antibody in the packing. And the SDS-containing Loading Buffer can denature the medium ligand. Anti-c-Myc Affinity Gel cannot be reused after denaturing elution.

5 Storage

4ºC, 2 years

Do not freeze

6 Precautions

1. Please resuspend the gel thoroughly before use.
2. Before using this product for IP (Immunoprecipitation) experiments, it is necessary to confirm the expression status of GFP tagged proteins in the sample.
3. To minimize protein degradation, protease inhibitor cocktails (MCE Cat. No. HY-K0010, HY-K0011) are highly recommended.
4. Do not use cell lysate samples containing DTT. DTT may cause the c-Myc antibodies on the gel to detach.
5. This product is for R&D use only, not for drug, household, or other uses.
6. For your safety and health, please wear a lab coat and disposable gloves to operate.