

UltraBio™ Anti-DYKDDDDK Agarose

U1508188

Storage: 2-8°C. Do not freeze.

Shipping: Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Size: 1mL/5 mL.

Introduction

Aladdin's UltraBio™ Anti-DYKDDDDK Agarose is produced by high-quality mouse anti-DYKDDDDK (Flag) monoclonal antibody conjugated to 4% agarose gel. DYKDDDDK tag, also known as FLAG tag, is a short peptide sequence that is commonly used as a protein tag to facilitate protein purification, detection, and localization. It is often fused to the N- or C-terminus of a protein of interest, allowing for easy identification and isolation of the protein using anti-FLAG antibodies. Therefore, this agarose can specifically bind DYKDDDDK fusion proteins in lysates, serum, ascites, or other samples from animals, plants, or microorganisms. This product is mainly used for immunoprecipitation (IP), co-immunoprecipitation (Co-IP), or purification of DYKDDDDK-tagged proteins or their protein complexes.

Aladdin UltraBio™ Anti-DYKDDDDK Agarose is stored in PBS containing 0.02% ProClin 300, with a settled gel to storage solution ratio of 1:1 (This product is a 50% gel suspension, containing 0.5 mL of settled agarose per milliliter. Our product specifications are based on total volume. Product information is detailed in the table below:

Parameter	Value/Description
Matrix	4% Agarose Microspheres
Ligand	Recombinant DYKDDDDK tag (Binds to Flag tag) Antibody
Isotype	Mouse IgG2a
Particle Size Range	45~165 µm
Antibody concentration	Approximately 3 mg DYKDDDDK tag Antibody /mL Settled Agarose
Maximum Pressure	0.3 MPa, 3 bar
Storage Conditions	0.01M PBS contains 0.02% ProClin 300, 2-8°C
Shelf Life	1 year

Instruction for use

1. Sample Preparation

- 1.1 Ensure the sample solution has appropriate ionic strength and pH before loading. Dilute the sample or cell culture supernatant with equilibration buffer, or dialyze the sample against equilibration buffer.

1.2 Clarify the sample by centrifugation or filtration through a 0.22 μm or 0.45 μm membrane to reduce impurities, improve purification efficiency, and prevent column clogging.

1.3 Preparation of protein sample. Lysis cells or tissues with appropriate lysis buffer. The following lysis buffers are recommended for preparing lysates from cells or tissues. For optimal results, Aladdin product C301898 Cell Total Protein Lysate for Western Blotting & Immunoprecipitation is the preferred choice for lysis of cell or tissue samples. Depending on the specific experimental needs, if necessary, Aladdin products RIPA Lysis Buffer (Strong), RIPA Lysis Buffer (Medium), or RIPA Lysis Buffer (Weak) may also be used for sample preparation. If using a self-prepared lysis buffer or one from another manufacturer, ensure that the pH of the lysis buffer is between 6 and 8. When using a self-prepared lysis buffer or one produced by other companies, ensure that the pH of the lysis buffer is 6-8.

2. Buffer Preparation

It is recommended to filter water and buffers through a 0.22 μm or 0.45 μm membrane before use.

Equilibration Buffer: 0.01 M PBS, pH7.4.

Wash Buffer: 0.01 M PBS, 0.05% Tween-20, pH7.4.

3. Immunoprecipitation (IP) Procedure

3.1 Resin Preparation: Add 40 μL of Anti-DYKDDDDK Agarose suspension (20 μL settled agarose) to a 1.5 mL tube. Centrifuge at $5000 \times g$ for 1 minute. Discard the supernatant.

3.2 Add 0.5 mL of Equilibration Buffer to resuspend the agarose (Place the agarose in the same buffer system as the target protein to protect the protein). Centrifuge at $5000 \times g$ for 1 minute. Discard the supernatant. Repeat this wash step once.

3.3 Add 300 μL of sample lysate to the prepared agarose from step 3.2. Mix and incubate on a tube rotator at room temperature for 3 hours. Centrifuge at $5000 \times g$ for 1 minute. Discard the supernatant.

3.4 Washing: Add 0.5 mL of Wash Buffer, resuspend the agarose, and mix gently. Centrifuge at $5000 \times g$ for 1 minute. Discard the supernatant. Repeat this wash step three more times. Ensure the removal of non-specifically adsorbed substances.

3.5 Denaturing Elution (SDS-PAGE): Add 100 μL of $1\times$ Loading Buffer to the agarose. Heat at 95°C for 5 minutes. Centrifuge at $5000 \times g$ for 1 minute, and load the supernatant directly onto an SDS-PAGE gel for analysis.

Note: The Standard loading buffer used in laboratories contains β -mercaptoethanol and DTT, which can break the disulfide bonds between the antibody heavy and light chains on the agarose. This method denatures the antibody, rendering the resin unusable for reuse.

Storage

Store at 4°C for up to 12 months. Do not store at -20°C or lower temperatures.

Matters needing attention

1. This product must be fully resuspended by inverting the tube several times prior to use.

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2. For immunoprecipitation assays, it is recommended to include both positive and negative controls.
3. Protein samples should be purified as soon as possible after collection and should always be placed at 4°C or on ice to minimize protein degradation or denaturation.
4. The non-specific binding capability of this product can be affected by high concentrations of DTT, mercaptoethanol, and guanidine hydrochloride, but it is applicable in Cell lysis buffer for Western and IP, RIPA Lysis Buffer, and NP-40 Lysis Buffer.
5. To effectively inhibit protein degradation, an appropriate amount of protease inhibitor cocktail can be added to the protein sample.
6. This product is for R&D only. Not for drug, household, or other uses.
7. For your safety and health, please wear a lab coat and disposable gloves during the operation.

A large, light blue, diagonal watermark of the Aladdin logo is positioned across the center of the page. It includes the stylized "a" with a yellow swoosh and the registered trademark symbol.