





## Anti-His Tag Mouse Monoclonal Antibody (5C3), Agarose

Cat #: A02050AGB

Size: 100µl /400µl /2ml

### Product Information

	<b>Product Name:</b> Anti-His Tag Mouse Monoclonal Antibody (5C3), Agarose		
	<b>Applications:</b> IP		<b>Isotype:</b> Mouse IgG1
	<b>Catalog Number:</b> A02050AGB		<b>Lot Number:</b> Refer to product label
	<b>Storage:</b> Store at 4°C. Avoid freeze-thaw or vortex.		<b>Note:</b> Contain sodium azide.
	<b>Formulation:</b> Over 2 mg of Antibody coupled to 1 ml of packed Agarose.		

**Product Description:** Anti-His Tag Agarose are prepared by covalently coupling Anti-His Tag Mouse Monoclonal Antibody to Agarose, useful for detection and capture of fusion proteins containing a His peptide sequence by commonly used immunoprecipitation procedures. The coupling technique is optimized to give a high binding capacity for His-tag protein.

**Storage Buffer:** 50% gel slurry suspended in PBS, pH 7.4, containing 0.02% Sodium Azide as preservative.

**Storage instructions:** Stable for one year at 4°C from date of shipment. Avoid freeze-thaw or vortex.

**Shipping:** Gel pack with blue ice.

**Note:** The product listed herein is for research use only and is not intended for use in human or clinical diagnosis. Suggested applications of our products are not recommendations to use our products in violation of any patent or as a license. We cannot be responsible for patent infringements or other violations that may occur with the use of this product.

### Suggested Procedure

#### Agaroses Preparation (Repeat for three times)

1. Transfer the appropriate amount of Agaroses (20 µL Agaroses for each sample) into a 1.5 ml microfuge tube .
2. Centrifuge at 4°C, 5000 *rpm* for 30s and remove the storage buffer.
3. Resuspend the Agaroses with 1 mL ice-cold 1×TBS.
4. Centrifuge at 4°C, 5000 *rpm* for 30s and remove the supernatant.

**Note:** For multiple samples, it is recommended to prepare the Agaroses first, and then aliquot into each microfuge tube.

#### Sample preparation

1. Collect  $2 \times 10^6$  cells and wash with PBS for three times.
2. Resuspend cells in 400 µL ice-cold lysis buffer [ 50 mM Tris (pH 7.5), 150 mM NaCl, 0.05% NP-40 ], then sonicate briefly (up to 10s).
3. Centrifuge 12000*g* for 5 minutes at 4°C and collect the supernatant.

## **Binding protein**

1. Add 400  $\mu$ L cell lysate to Agaroses.
2. Mix thoroughly and incubate at 4°C for 1-2 h.

## **Wash (Repeat 3-5 times until OD280 of the supernatant is lesser than 0.05)**

1. Centrifugate at 4°C, 5000 *rpm* for 30s and remove the supernatant to collect the mixture.
2. Resuspend the mixture with 1 mL ice-cold 1×TBS and incubate it at 4°C for 1-3 minutes.
3. Centrifugate at 4°C, 5000 *rpm* for 30s and remove the supernatant. (Or save the supernatant for further analysis)

**Note:** Avoid losing Agaroses during wash step.

## **Elution**

For SDS-PAGE detection -- Add 50  $\mu$ L 1×protein sample buffer to the above obtained precipitate, and then boil for 5 min. Cool to room temperature.

For other assay -- Add 2 volumes (vs Agaroses volume) elution buffer [ 0.1 M -0.2 M Glycine pH 2.5-3.1 (or 0.1 M citric acid, pH 2.5-3.1 or 2.5% Acetic Acid) ] to the above obtained precipitate, and incubate at least 2 minutes to collect elution fraction. (Repeat for three times).