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High-Affinity Iodoacetyl Agarose

1 Contents

Components	HY-K0219-5 mL	HY-K0219-10 mL	HY-K0219-50 mL
High-Affinity Iodoacetyl Agarose (Settled Resin)	5 mL	10 mL	50 mL

2 General Information

MCE High-Affinity Iodoacetyl Agarose, a 4% highly cross-linked agarose reagent coupled with a derivative of iodoacetic acid, reacts specifically with free sulfhydryls of sulfhydryl-containing peptides, proteins or other ligands and form stable thioether linkage. The agarose is ideal for conjugating sulfhydryl-containing peptide or protein for subsequent affinity purification. 1 mL of agarose can immobilize approximately 1 mg sulfhydryl-containing peptides of 7 amino-acids.

3 Characteristics

Matrix Spherical	4% cross-linked agarose
Mean Bead Diameter	95 μm
pH Stability	pH 5-10
Binding Capacity	>3 mg Goat IgG/mL settled resin
Storage Solution	50% slurry in 1× PBS containing 20% ethanol

4 General Protocol

1. Buffer Preparation

Coupling Buffer	50 mM Tris•HCl, 5 mM EDTA-Na, pH 8.5
Block Buffer	50 mM L-Cysteine hydrochloride (HY-Y0337A) in Coupling Buffer
Storage Buffer	1× PBS containing 20% ethanol
Binding/Wash Buffer	1× PBS, pH 8.0
Elution Buffer	0.1 M Glycine, pH 2.0-2.5
Neutralization Buffer	1 M Tris-HCl, pH 8.5

Buffer filtration with 0.45 μm filter is recommended.

2. Coupling Sulfhydryl-containing Sample

(1) Sample Preparation. Dissolve the sulfhydryl-containing peptide or protein sample in Coupling Buffer at the concentration of 1 mg/ml.

(2) Load of High-Affinity Iodoacetyl Agarose. Completely suspend the agarose by gently inverting the bottle several times. Transfer an appropriate-sized slurry to the column and allow the agarose to settle down and the storage buffer to drain from the column.

(3) Equilibrate the column with 3× bed volumes of Coupling Buffer with a flow speed of about 1 mL/min. Repeat this equilibration step two more times.

(4) Add appropriate-sized sulfhydryl-containing sample to the column. Sequentially cap the bottom and top and incubate on a shaker at room temperature for 30 minutes. After incubation, place the column upright in the stand, remove the cap and allow the agarose to settle and buffer to drain.
Note: Approximately 1 mL sample solution can be applied to 1 mL High-Affinity Iodoacetyl Agarose.
(5) Wash the column with approximately 3× bed volumes of Coupling Buffer with a flow speed of about 1 mL/min. Collect the flow through and wash to determine the coupling efficiency by comparing the concentrations of the starting sample before and after coupling. The concentration can be measured by absorbance at 280nm or by concentration of the free sulfhydryl group.
(6) Add Block Buffer (1 mL Block Buffer can be applied to 1 mL High-Affinity Iodoacetyl Agarose). Sequentially cap the bottom and top and incubate on a shaker at room temperature for 30 minutes. After incubation, place the column upright in the stand, remove the cap and allow the agarose to settle and buffer to drain.
(7) Storage. For immediate use, go to Step 3. For future use, wash the column with 3× bed volumes of Binding/Wash Buffer. The agarose can be stored in 1× bed volume of Storage Buffer at 4ºC.

3. Antidody Purification

(1) Equilibrate the column with 5× bed volumes of Binding/Wash Buffer with a flow speed of about 1 mL/min.
(2) Apply appropriate-sized antibody to the column with a flow speed of about 0.5 mL/min. Collect and save the flow-through for analysis.
Note: Binding capacity is flow rate- and protein-dependent. Higher flow rates will decrease production time, but may result in losing a small portion of the target antibody.
(3) Wash the column with approximately 30-50× bed volumes of Binding/Wash Buffer with a flow speed of about 1.5 mL/min or until the absorbance at 280 nm is stable. If desired, save supernatant for downstream analysis.
(4) Elute with approximately 5-10× bed volumes of Elution Buffer with a flow speed of about 1 mL/min. Collect the eluate and immediately neutralize with Neutralization Buffer (1/10 volume of total eluate).
(5) Analyze the target antibody by SDS-PAGE, along with fractions collected from different steps if necessary.
(6) Regenerate the column with 5× bed volumes of Binding/Wash Buffer. Store the agarose in 1× bed volume of Storage Buffer at 4°C. Do not freeze the agarose.

5 Storage

Store at 2-8ºC, and is stable for at least 2 years.
Do not dry or freeze.

6 Precautions

1. Do not dry or freeze the agarose.
2. This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.
3. For your safety and health, please wear a lab coat and disposable gloves to operate.

7 Troubleshooting

Problem	Possible Cause	Solution
Highly backpressure	Sample was highly particulate.	Centrifuge or filter (0.45 μm) before use.
	Gas presents in buffers or sample on the column, which results in blockage of gel pores with microscopic air bubbles.	Degas buffers and remove air bubbles from column.
Protein/peptide precipitates in Coupling Buffer	Protein/peptide was not soluble in Coupling Buffer.	Coupling Reaction is compatible with 30% DMSO or 6M Guanidine•HCl, if necessary, add these reagents to the coupling reaction to maintain solubility of the protein/peptide.
Low coupling efficiency	Sulfhydryl groups are not free, they are oxidized.	Reduce protein/peptide with DTT or TCEP and proceed immediately to coupling procedure after desalting to prevent reformation of disulfide bonds.
Antibody of interest purified, but it is denatured	The antibody is sensitive to low-pH elution buffer.	Neutralize the eluted fractions with Neutralization Buffer immediately after elution.