HyperChrom Anti-DYKDDDDK Agarose

Introduction

HyperChrom Anti-DYKDDDDK Agarose is an affinity chromatography medium based on a highly cross-linked agarose gel. The Flag tag is a polypeptide fragment (DYKDDDDK) consisting of eight hydrophilic amino acids that is localized to the surface of the fusion protein and is therefore more readily bound to antibodies and broken down by enterokinases. This chromatography medium can be used for experiments such as purification and immunoprecipitation (IP) of fusion proteins tagged with DYKDDDDK.

HyperChrom Anti-DYKDDDDK Agarose is based on a 4% agarose gel that reduces non-specific binding of heteroproteins. It can be regenerated and reused, generally 3-10 times.

Components and Storage

Components	1 mL	5 mL	25 mL	25 mL	100 mL	500 mL
HyperChrom Anti-DYKDDDDK Agarose	1 mL	5 mL	10 mL	25 mL	50 mL	100 mL
Shipping: Blue ice	**Shelf life: Store at 4°C for 2 years**					

Product parameters

HyperChrom Anti-DYKDDDDK Agarose parameters

Name	Description
Chromatography media type	Affinity chromatography media
Ligation	Anti-DYKDDDDK
Scaffolding	4% highly cross-linked agarose
Average particle size	45-165 μm
Dynamic load	>1 mg DYKDDDDK protein/mL chromatography medium
Pressure-resistant	0.1 MPa
Storage solution	1×PBS, 0.02% NaN_3

Protocol

1. Preparation of buffers (it is recommended to filter the water and buffer with a 0.22 μm or 0.45 μm filter before use).

- Balance/Wash Buffer: 50 mM Tris，0.15 M NaCl，pH7.4
- Acid Elution Buffer: 0.1 M glycine HCl，pH3.0
- Competitive Elution Buffer：50 mM Tris，0.15 M NaCl，100-500 μg flag peptides/mL，pH7.4
- Neutralization Buffer：1 M Tris-HCl，pH8.0

2. Sample preparation

Before loading the column, make sure that the sample solution has the appropriate ionic strength and pH value, and the sample or cell culture medium can be diluted with the Balance Buffer, or dialysed with the Balance Buffer; Samples are recommended to be centrifuged or filtered through a 0.22 μm or 0.45 μm filter membrane prior to loading to reduce impurities, improve protein purification efficiency, and prevent clogging of the column.

3. Sample purification

3.1 Column chromatography

a) Resin preparation: HyperChrom Anti-DYKDDDDK Agarose is loaded into a suitable chromatography column and equilibrated with 5 column volumes of Balance Buffer so that the resin is in the same buffer system as the protein of interest.

b) Dispensing: Samples are added to a balanced HyperChrom Anti-DYKDDDDK Agarose to collect the effluent, which can be loaded repeatedly to increase binding efficiency.

c) Wash and collect: Wash with 10 times the column volume of Wash Buffer to remove non-specific adsorption of miscellaneous proteins and collect the wash solution.

d) Elution:

- Acid elution: Use 5 column volumes of Acid Elution Buffer, add one-tenth of the elution volume of Neutralization Buffer to the elution component, adjust the pH to 7.0-8.0, and manage the collection. Note: Immediately after acid elution, the resin should be equilibrated with a balanced solution, and HyperChrom Anti-DYKDDDDK Agarose should not be in the eluent for more than 20 minutes.
- Competitive elution: Elute using 5 column volumes of Competitive Elution Buffer. In charge of collection.

e) Packing regeneration: Regenerate the packing using 3 column volumes of elution buffer, then equilibrate to neutral with Balance Buffer.

f) Packing material preservation: The washed resin is stored in PBS solution containing 0.02% sodium azide at 2-8°C.

3.2 Static adsorption

a) Packing preparation: take appropriate amount of HyperChrom Anti-DYKDDDDK Agarose and add it to the chromatographic column to flow into the protective fluid. Add 5 times the volume of the column Balance Buffer cleaning.

b) Loading and incubation: Add the sample solution and incubate at 4°C or room temperature for at least 30 minutes (no magnetic stirring) to ensure that the packing material is well mixed with the sample solution.

c) Separation: After incubation, centrifuge the packing mixture (5000×g for 1 min) or filter to collect the resin.

d) Equilibration: The resin is loaded into the chromatography column and washed with the balance solution until UV stable.

e) Elution:

Elution with Acid Elution Buffer or Competitive Elution Buffer, refer to 3.1.d.

f) Filler regeneration: Ref. 3.1.e.

g) Filler preservation: Ref. 3.1.f.

3.3 Immunoprecipitation procedure

a) Packing preparation: Take 40 μL of HyperChrom Anti-DYKDDDDK Agarose (column volume 20 μL) mixture and add it to 2 mL centrifuge tube, centrifuge 5000×g for 1 min, then discard the supernatant.

b) Equilibration: 0.5 mL of Balance Buffer was added to the resin, the resin was suspended (the resin was in the same buffer system as the target protein, which played a role in protecting the protein), centrifuged at 5000×g for 1 min, and the supernatant was aspirated. Repeat once.

c) Sampling and incubation: Add 200-1000 μL of sample lysate to the processed packing material, mix well, and place it in an inverted mixer at room temperature to gently flip the centrifuge tube to promote full contact and adsorption between the sample and the packing material for at least 1 h at room temperature. Centrifuge at 5000×g for 1 min, and aspirate and discard the supernatant.

d) Washing: Add 0.5 mL of Wash Buffer, suspend the packing, mix gently, centrifuge at 5000×g for 1 min, and aspirate the supernatant. Repeat three more times. Make sure to remove non-specific adsorption.

e) Sample elution: Different elution methods can be selected according to the needs of later detection.

- Acid elution: Add 100 μL of Acid Elution Buffer and suspend the packing. Incubate at room temperature for 5 min, centrifuge at 5000×g for 1 min. Carefully remove the supernatant, do not aspirate the filler, and neutralize with Neutralization Buffer. Eluted samples are stored at 4 °C and stored at -20°C for a long time.
- Competitive elution: Add 100 μL of Competitive Elution Buffer to elute. Incubate at room

temperature for 30 min, centrifuge at 5000×g for 1 min. Carefully remove the supernatant without suctioning the filler. Eluted samples are stored at 4°C and stored at -20°C for a long time.

- Denaturing elution: Add 20μL of 2× Loading Buffer to each tube and heat at 95°C for 5 min. After centrifugation at 5000×g for 1 min, the supernatant was absorbed for SDS-PAGE electrophoresis.

***Note:** Laboratory routine protein Loading Buffer contains β-mercaptoethanol and DTT, which can disconnect the heavy and light chains of antibodies in the filler. Sample buffers containing SDS can denature Anti-DYKDDDDK antibodies, and HyperChrom Anti-DYKDDDDK Agarose cannot be reused after elution.

frequently asked questions

1. HyperChrom Anti-DYKDDDDK Agarose compatibility:

Reagent Name	Maximum tolerated concentration	Notes
β-mercaptoethanol	10 mM	It should be avoided during purification and cannot be recycled if used in IP
DTT	80 mMM	
SDS	-	
Tween-20	5%	Excessive concentrations can affect the binding efficiency of the tagged protein
Triton X-100	5%	
NP-40	4%	
Guanidine hydrochloride	0.3 M	Too high a concentration can denature the antibody
Urea	1.5 M	
EDTA	5 mM	Too high EDTA can reduce protein recovery
Sodium chloride	1 M	Reduces non-specific adsorption
Glycerol	20%	Excessive concentration can affect tag protein binding

2. Common problems and solutions

Question	Reasons	Suggestion
There is no protein of interest in the eluate	There are no tag-and-fusion proteins in the sample	Western detection for flag-tagged fusion proteins prior to purification.
	The protein of interest is unstable	Use fresh samples, handle them at low temperatures, and add protease inhibitors.
	The expression of the target protein is too low	Optimize protein expression, increase sample loading, and reduce NaCl concentration.
There is a protein of interest in the flowthrough	Protein load overload	Reduce the sample volume or increase the amount of resin.
	The incubation binding time is too short	Extend the binding time of samples and resins.
	The tag of the recombinant protein is not exposed	A low concentration of denaturant was added, and dialysis was performed before sample loading.

	The reagents in solution are not compatible	Samples are subjected to dialysis prior to loading.
The background is too mixed	Non-specific adsorption	Reduce the amount of sample loaded
	Insufficient washing	Increase the number of washing and increase the concentration of salt ions in the washing solution

Notes

1. It is recommended that the buffers and protein solutions used be filtered through a 0.22 μm or 0.45 μm filter and then used as resins.
2. This product is for scientific research use only.

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