TiO_2 Mag Sepharose

TiO_2 Mag Sepharose™ is available in the following formats (Instructions for use are included):

- 1 × 500 µl TiO_2 Mag Sepharose, 20% medium slurry
- 4 × 500 µl TiO_2 Mag Sepharose, 20% medium slurry

Purpose

TiO_2 Mag Sepharose is magnetic beads designed for enrichment of phosphopeptides from tryptic digested protein samples and addresses the need for easy small-scale preparation of protein samples prior to analyses such as mass spectrometry (MS), and liquid chromatography mass spectrometry (LC-MS).

Intended use

This product is intended for research only, and should not be used in any clinical or in vitro procedures for diagnostic purposes.

Table of contents

1 Principle

TiO_2 Mag Sepharose is based on titanium dioxide (TiO_2) chromatography and is designed for magnetic separation technique. TiO_2 has a high affinity to phosphorylated peptides, which makes TiO_2 Mag Sepharose useful for selective enrichment of phosphopeptides.

Used together with 1.5 ml Eppendorf tubes and a magnet rack, for example MagRack 6 (see Section 6), the magnetic beads are easily separated from the liquid phase during the different steps of the enrichment protocol.

2 Advice on handling

Note: *TiO_2 Mag Sepharose is intended for single use only.*

General handling

Dispensing the medium slurry

- Use a wide pipette tip or a regular pipette tip with the end cut off.
- Use 1.5 ml Eppendorf tubes.
- Prior to dispensing the medium slurry, make sure it is homogeneous by vortexing or by repeated manual inversion of the vial.
- When the medium slurry is resuspended, pipette immediately the required amount of medium slurry into the Eppendorf tube.
- Repeat the resuspension step between every pipetting from the medium slurry vial.

Handling of liquid

- Use the magnetic rack with the magnet in place for each liquid removal step.
- Before application of liquid, wash buffer, elution buffer etc., remove the magnet from the magnetic rack.
- After addition of liquid, allow resuspension of the beads by vortex or manual inversion of the Eppendorf tube.
- When processing multiple samples, manual inversion of the magnetic rack is recommended.

Incubation steps

- During incubation steps, make sure the gel beads are well resuspended and kept in solution by use of a mixer suitable for 1.5 ml Eppendorf tubes.
- If needed, use a micro centrifuge to remove liquid from the lid, especially before the elution step.
- All incubations should be performed at room temperature.

Sample pretreatment

- For complex samples, such as cell lysate digests, it is recommended to perform a desalting step by use of for example a RPC/C18 cartridge or similar for efficient phosphopeptide enrichment.
- Dilute your sample with minimum 4 volumes of binding buffer or dissolve lyophilized sample in binding buffer.
- Keep sample volumes small, preferably max 100 µl, however up to 250 µl may be used.

Recommended buffers

Note: *Use high-purity water and chemicals for buffer preparation.*

Buffer	Composition
Binding buffer	• 1 M glycolic acid in 80% acetonitrile, 5% trifluoroacetic acid
Wash buffer	• 80% acetonitrile, 1% trifluoroacetic acid
Elution buffer	• 5% ammonium hydroxide, pH ~ 12

Analysis

Eluates must be evaporated or neutralized with formic acid or trifluoroacetic acid before analysis with MALDI-ToF. Suitable solvent for evaporated samples is 20% acetonitrile acidified with 0.1% trifluoroacetic acid.

For LC-MS analysis using reversed phase chromatography (RPC) the eluates must firstly be evaporated and resuspended in formic acid to a final concentration of 0.1%.

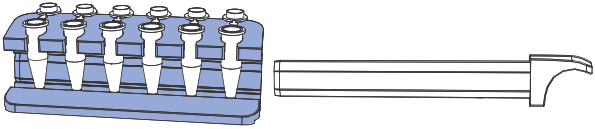
3 Safety precautions

Always use personal protection devices like gloves and safety glasses when handling TiO_2 Mag Sepharose.

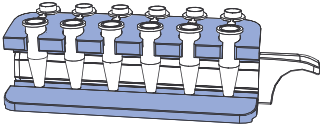
4 Protocol

General magnetic separation step

1 *Remove* the magnet before *adding* liquid.



2 *Insert* the magnet before *removing* liquid.



Protocol

1 Magnetic bead preparation

A Add 50 µl medium slurry to a 1.5 ml Eppendorf tube (micro tube) using a wide pipette tip or a regular pipette tip with the end cut off.

B Place the Eppendorf tube in the magnetic rack, for example MagRack 6.

C Remove the storage solution.

2 Equilibration

A Add 500 µl binding buffer.

B Resuspend the medium by manual inversion a few times.

C Remove the liquid.

3 Sample application

A Add sample (50 µl to 250 µl) as prepared according to Section 2.

B Resuspend the beads and incubate for 30 minutes in a mixer suitable for 1.5 ml Eppendorf tubes.

C Remove the liquid.

4 Wash 1

A Add 500 µl binding buffer.

B Resuspend the medium by manual inversion a few times.

C Remove the liquid.

5 Wash 2 and 3 (perform this step 2 times totally)

A Add 500 µl wash buffer.

B Resuspend the medium by manual inversion a few times.

C Remove the liquid.

6 Elution

A Elute the sample by adding 50 µl elution buffer. Incubate for 5 minutes in a mixer suitable for 1.5 ml Eppendorf tubes.

B Collect the eluate.

C Repeat this step once and pool the eluted fractions.

5 Characteristics

Matrix	Paramagnetic spherical, highly cross-linked agarose particles including TiO_2
Medium	TiO_2 Mag Sepharose
Particle size	37 to 100 µm
Working temperature	Room temperature
Storage solution	20% ethanol
Storage temperature	+4°C to +30°C

6 Ordering Information

Products	Quantity	Code No.
TiO_2 Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-10
TiO_2 Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-77

Related products	Quantity	Code No.
Protein A Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-06
Protein A Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-78
Protein G Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-08
Protein G Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-79
NHS Mag Sepharose	1 × 500 µl 20% medium slurry	28-9440-09
NHS Mag Sepharose	4 × 500 µl 20% medium slurry	28-9513-80
MagRack 6	1	28-9489-64
Phos SpinTrap™ Fe	1	28-9298-81
Nuclease Mix	0.5 ml	80-6501-42
Protease Inhibitor Mix	1 ml	80-6501-23

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