TiO\textsubscript{2} Mag Sepharose

TiO\textsubscript{2} Mag Sepharose\textsuperscript{TM} is available in the following formats (Instructions for use are included):

- 1 × 500 μl TiO\textsubscript{2} Mag Sepharose, 20% medium slurry
- 4 × 500 μl TiO\textsubscript{2} Mag Sepharose, 20% medium slurry

Purpose

TiO\textsubscript{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.
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1 Principle

TiO₂ Mag Sepharose is based on titanium dioxide (TiO₂) chromatography and is designed for magnetic separation technique. TiO₂ has a high affinity to phosphorylated peptides, which makes TiO₂ 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

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binding buffer</td>
<td>1 M glycolic acid in 80% acetonitrile, 5% trifluoroacetic acid</td>
</tr>
<tr>
<td>Wash buffer</td>
<td>80% acetonitrile, 1% trifluoroacetic acid</td>
</tr>
<tr>
<td>Elution buffer</td>
<td>5% ammonium hydroxide, pH ~ 12</td>
</tr>
</tbody>
</table>

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

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 TiO₂ 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

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Paramagnetic spherical, highly cross-linked agarose particles including TiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>TiO₂ Mag Sepharose</td>
</tr>
<tr>
<td>Particle size</td>
<td>37 to 100 μm</td>
</tr>
<tr>
<td>Working temperature</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Storage solution</td>
<td>20% ethanol</td>
</tr>
<tr>
<td>Storage temperature</td>
<td>+4°C to +30°C</td>
</tr>
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</table>
### 6 Ordering Information

<table>
<thead>
<tr>
<th>Products</th>
<th>Quantity</th>
<th>Code No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO₂ Mag Sepharose</td>
<td>1 × 500 μl 20% medium slurry</td>
<td>28-9440-10</td>
</tr>
<tr>
<td>TiO₂ Mag Sepharose</td>
<td>4 × 500 μl 20% medium slurry</td>
<td>28-9513-77</td>
</tr>
</tbody>
</table>

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