AMP Affinity Test Kit
Screening Kit for the purification of AMP binding proteins

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Amount</th>
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<tbody>
<tr>
<td>AK-107</td>
<td>1 Kit</td>
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</tbody>
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For research use only!

Shipping: shipped at 4 °C
Storage Conditions: store at 4 °C
Additional Storage Conditions: do not freeze
Shelf Life: 12 months after date of delivery

Description:
A characteristic of many proteins is their ability to bind specific small molecules (ligands) non-covalently with high affinity. This protein-ligand interaction can be used for rapid purification of a protein by affinity chromatography. In this technique, a ligand (e.g. a nucleotide) is immobilized onto the surface of a matrix (e.g. Agarose), which is incubated with a protein mixture to be purified. The protein of interest will bind to its ligand whereas other contaminants will not. These contaminants can be washed off, and the protein of interest can be eluted by an excess of free ligand in the elution buffer.

A ligand commonly used for this technique is AMP (Adenosine-5’-monophosphate) that is suitable (when immobilized onto an agarose matrix) for the purification of various NADH/NADPH-dependent dehydrogenases, ATP-dependent and AMP-binding enzymes as well as HIT family proteins (Tab. 1).

There is however, a fundamental problem with using AMP in affinity chromatography: For attachment to a matrix AMP needs to be chemically modified with a linker (Fig. 1). This linker may interfere with the protein-AMP interaction and thereby reduce the binding. This problem can usually be circumvented by attaching AMP at a different position at the adenine base, the sugar or the phosphate moiety. Each of these linkage strategies has a characteristic effect upon protein-AMP interactions (Fig. 1).

The AMP Affinity Test Kit contains a set of 4 typical AMP-Agarose chromatography materials as well as the unmodified (blank) Agarose as a negative control.

6AH-AMP-Agarose and 8AHA-AMP-Agarose contain AMP that is immobilized via the adenine base but varies by the actual position of the linker (C₆ and C₈, respectively). αAH-AMP-Agarose and EDA-AMP-Agarose are phosphate and sugar modified derivates, respectively (Fig. 2).

With these four materials, the ideal material for purification of a particular protein of interest can be identified in a simple screening experiment.

Content:
- 250 µl α-Amino-hexyl-AMP-Agarose (αAH-AMP-Agarose) (AC-158)
- 250 µl 8-[(6-Amino)hexyl]-amino-AMP-Agarose (8AHA-AMP-Agarose)
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Agarose) (#AC-156)
- 250 µl N6-(6-Amino)hexyl-AMP-Agarose (6AH-AMP-Agarose) (#AC-145)
- 250 µl 2’/3’-EDA-AMP-Agarose (EDA-AMP-Agarose) (#AC-157)
- 250 µl Agarose (blank) (#AC-001)

Each Agarose is pre-swollen in 20% ethanol, 0.1 M Tris HCl pH 7.5

Properties of AMP-Agaroses

| Property                      | 45 - 165 µm | 5 µmol/ml Agarose for 6AH-AMP-, 6AH-AMP-, 8AHA AMP- and EDA-AMP-Agarose | 4 - 9 | 7.5 | Stable to all solutions commonly used in gel filtration including 8 M urea and 6 M guanidine hydrochloride. | Not stable in organic solvents! |

General experimental remarks

The optimal purification procedure varies depending on the protein of interest. Williams (1999) provides general background information on the usage of AMP agaroses that may be used as a starting point for the set up and optimization of your individual purification procedure. The following proteins have been successfully purified with AMP Agaroses:

Table 1: AMP-Agaroses purified proteins

<table>
<thead>
<tr>
<th>NADH/NADPH-dependent enzymes</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Glutamate dehydrogenase</td>
<td>Brodelius (1997)</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>Petit (1981)</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>Nealon (1979)</td>
</tr>
<tr>
<td>S-Nitrosoglutathione (GSNO) Reductase</td>
<td>Liu (2001)</td>
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ATP-dependent & AMP-binding enzymes

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<tr>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>aminoacyl-tRNA synthetases</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
</tr>
<tr>
<td>Deoxythymidine-5' Phosphotransferase</td>
</tr>
<tr>
<td>HIT protein family</td>
</tr>
<tr>
<td>Hint2</td>
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</tbody>
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Related Products:
PBS Tablets, #AK-102P
200 mM Sodium Orthovanadate, activated, #AK-102V
100X Protease Inhibitor Mix, #AK-102I

Selected References:
Bretes et al. (2013) Hint2, the mitochondrial nucleoside 5'-phosphoramidate hydrolase; properties of the homogeneous protein from sheep (Ovis aries) liver. Acta Biochim. Pol. 60 (2):249.
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