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Technical Information about Sp-6-AE-cAMPS-Agarose

Gel for affinity chromatography of various cyclic nucleotide-responsive proteins

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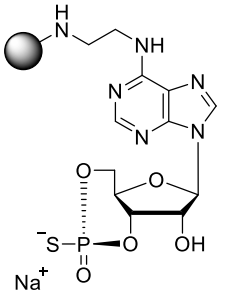

Abbreviation: **Sp-6-AE-cAMPS-Agarose**

BIOLOG Cat. No.: A 109

Description: In Sp-6-AE-cAMPS-Agarose the protein kinase A activator Sp-cAMPS (BIOLOG Cat. No. A 003) has been immobilized on agarose by an aminoethyl spacer attached to position N^6 of the ligand.

Properties: The ligand of this gel represents an immmobilized form of the cyclic AMP agonist Sp-cAMPS. Free Sp-cAMPS is only very slowly metabolized by corresponding phosphodiesterases and thus represents an extraordinary stable ligand. In addition, its reduced binding affinity towards protein kinase A compared to cAMP results in potentially milder desorption conditions in affinity chromatography. The gel can be used for affinity chromatography of various cyclic nucleotide-responsive proteins such as protein kinases, phosphodiesterases and others.
In case the target protein is absorbed too strongly on common cAMP agaroses, Sp-6-AE-cAMPS-Agarose can be used as alternative due to the reduced binding affinity of phosphorothioate-modified cyclic nucleotides to most receptors.
This type of gel is also offered with a longer spacer (Sp-6-AH-cAMPS-Agarose, BIOLOG Cat. No. A 108). The free ligand Sp-6-AE-cAMPS (Cat. No. A 092) is available as well. For immobilized Sp-cAMPS or Sp-cGMPS with other spacer types, attachment positions, different immobilization techniques or on other gels, please inquire or visit our website www.biolog.de.

Specification: Suspension in 30 mM Na_2HPO_4 buffer (pH 7). Ligand density: approximately 6 µmol/ml of settled gel. UV: λ_{max} 266 nm/suspension in glycol.

Stability and Storage: Sp-6-AE-cAMPS-Agarose has sufficient stability for chromatography at ambient temperature and does not need special care during handling or shipment. Nevertheless, for longer storage periods the gel should be kept in the refrigerator at +4 - +8 °C. **Storage buffer should contain 0.1% sodium azide for prevention of microbial growth.**

Chromatography: After equilibration with about 10 column volumes of starting buffer the affinity column is loaded with the protein solution, e.g. at 50-200 µl/min. In order to elute other nucleotide-dependent proteins unspecifically bound, the column is washed, e.g. with 1 mM 5'-AMP. For elution of E. coli nucleoside diphosphate kinase it is recommended to wash the column with 10 mM ADP/20mM $MgCl_2$.
Elution of the target protein is performed by a cyclic nucleotide gradient up to 40 mM. For elution of phosphodiesterases it could be advisable to use the highly hydrolysis-resistant Sp-cAMPS or the stable Rp-cAMPS.
Suitable buffer systems for your special application have to be tested, but phosphate should be not optimal since one essential affinity interaction of cyclic nucleotides towards their target receptors is the cyclic phosphate.
Regeneration can be achieved by a combination of up to 100 mM cAMP and buffer salts or 8 M urea.

Toxicity and Safety: Please keep in mind that the *in vivo* properties of this compound are not sufficiently characterized up to now. Avoid skin contact or ingestion and allow only trained personnel to work with it.
Our products are designed, developed and sold for research purposes only. They are intended for *in vitro* and non-human *in vivo* laboratory applications. Any other use requires approval of health authorities.
Not for drug, household or related uses!

Selected References for cAMP Affinity Chromatography:

Hanke, S.E.; Bertinetti, D.; Badel, A.; Schweinsberg, S.; Genieser, H.G.; Herberg, F.W., *N. Biotechnol.*, **28**, 294 - 301 (2011): "Cyclic Nucleotides as Affinity Tools: Phosphorothioate cAMP Analogues Address Specific PKA Subproteomes"

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