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Technical Information about Sp-2-AHA-cAMPS-Agarose

Gel for affinity chromatography of cAMP-phosphodiesterases and protein kinases

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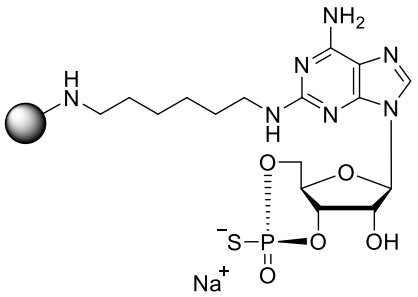

Abbreviation: **Sp-2-AHA-cAMPS-Agarose**

BIOLOG Cat. No.: A 069

Description: The protein kinase A activator Sp-cAMPS has been immobilized on agarose by an aminohexylamino spacer attached to position 2 of the ligand. Synthesis and distribution is protected by patent DE 3802865.4, licensed to BIOLOG Life Science Institute.

Properties: The ligand of this gel represents an immmobilized form of the cyclic AMP agonist Sp-cAMPS. Sp-2-AHA-cAMPS-Agarose is a gel for affinity chromatography of various cAMP-responsive proteins such as protein kinases or phosphodiesterases.
Since free Sp-cAMPS is only extremely slowly hydrolyzed by mammalian phosphodiesterases, the gel can be used for phosphodiesterase purification and isozyme pattern analysis as well. Immobilized Sp-cAMPS can also be used as a stable general ligand with reduced affinity for various nucleotide binding proteins since PDE degradation, the first metabolic step necessary in cyclic nucleotide metabolism, is blocked.
Immobilized Sp-cAMPS is also available with altered attachment positions of the spacer (position 8: Cat. No. A 013, position 2': Cat. No. A 050); for other spacer types, attachment positions or gels please inquire.
In case the target protein is absorbed too strongly on common cAMP agaroses, Sp-2-AHA-cAMPS-Agarose can be used as alternative due to the reduced binding affinity of phosphorothioate-modified cyclic nucleotides to most receptors. Also, the column can be useful for purification of cGMP receptors in case the protein sticks too tightly to a corresponding gel with immobilized cGMP.

Related Products: For immobilized cAMP or cGMP with other spacer types, attachment positions, different immobilization techniques or on other gels, please inquire. The free ligand Sp-2-AHA-cAMPS is offered separately as well (Cat. No. A 068).

Specification: Suspension in 30 mM Na_2HPO_4 buffer (pH 7). Ligand density: approximately 6 µmol/ml of settled gel. UV: λ_{max} 258 nm/ suspension in glycol.

Purity: The purity of the ligand to be attached is better than 98% (HPLC).

Stability and Storage: Sp-2-AHA-cAMPS-Agarose has sufficient stability for chromatography at ambient temperature and does not need special care during handling or shipment. Nevertheless for longer storing periods the gel should be kept in the refrigerator at +4 - +8°C since desulfurization yielding immobilized cAMP can occur slowly (about 0.1 % week/20°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!

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