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# **Technical Information about 8-AET-c-diAMP-Agarose**

## Gel for affinity chromatography of c-diAMP-responsive proteins

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#### Abbreviation:

8-AET-c-diAMP-Agarose

### BIOLOG Cat. No.: A 234

**Description:** In 8-AET-c-diAMP-Agarose 8-AET-c-diAMP (8-(2-Aminoethylthio)-cyclic diadenosine monophosphate, Cat. No. A 220) has been immobilized as an affinity ligand.

**Properties:** 8-AET-c-diAMP-Agarose can be useful for affinity chromatography of c-diAMP-responsive proteins. c-diAMP (Cat. No. C 088) is a purine-based signalling nucleotide in bacteria.

**Specification:** Suspension in 30 mM Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 7). Ligand density: approximately 1  $\mu$ mol/ml of settled gel. UV:  $\lambda_{max}$  267 nm/suspension in glycol.

**Stability and Storage:** 8-AET-c-diAMP-Agarose has sufficient stability for chromatography at ambient temperature. Nevertheless, for longer storage periods the gel should be kept in the refrigerator at + 4 - + 8 degrees Celsius. Storage buffer should contain 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. In order to elute proteins unspecifically bound, the resin is washed, e.g. with 10 mM GTP, 1 mM ATP, 0.25 mM cAMP and/or 0.25 mM cGMP. Elution of c-diAMP-binding proteins can be performed with free c-diAMP (e.g. 100 - 500 µM). An alternative protocol for elution using 5 mL of 9 M urea in PBS containing 1% (vol/vol) Triton X-100 can be found in Bai et al. (2014). Suitable buffer systems for your special application have to be tested. Regeneration of the agarose may be achievable by incubation with 8 M urea and subsequent washing with a suitable buffer.

**Toxicity and Safety:** Please keep in mind that the *in vivo* properties of this product 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 8-AET-c-diAMP-Agarose:** 8-AET-c-diAMP-Agarose is a new product which has been synthesized by BIOLOG Life Science Institute for the first time. There are no corresponding references available at present.

#### Selected Reference for the Related Product 2'-AHC-c-diAMP-Agarose (Cat. No. A 183):

Bai, Y.; Yang, J.; Zarrella, T.M.; Zhang, Y.; Metzger, D.W.; Bai, G., *J. Bacteriol.*, **196**, 614 - 623 (2014): "Cyclic di-AMP Impairs Potassium Uptake Mediated by a c-di-AMP Binding Protein in Streptococcus pneumonia"



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#### Selected References for the Bacterial Second Messenger c-diAMP (Cat. No. C 088):

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Corrigan, R.M.; Abbott, J.C.; Burhenne, H.; Kaever, V.; Gründling, A., *PLoS Pathog.*, **7**, e1002217 (2011): "c-di-AMP is a New Second Messenger in Staphylococcus aureus with a Role in Controlling Cell Size and Envelope Stress"

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Gomelsky, M., Mol. Microbiol., 79, 562 - 565 (2011): "cAMP, c-di-GMP, c-di-AMP and now cGMP: Bacteria use Them All!"

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Tchigvintsev, A.; Xu, X.; Singer, A.; Chang, C.; Brown, G.; Proudfoott, M.; Cui, H.; Flick, R.; Anderson, W.F.; Joachimiak, A.; Galperin, M.Y.; Savchenko, A.; Yakunin, A.F., *J. Mol. Biol.*, **402**, 524 - 538 (2010): "Structural Insight into the Mechanism of c-di-GMP Hydrolysis by EAL Domain Phosphodiesterases"

Woodward, J.J.; lavarone, A.T.; Portnoy, D.A., Science, **328**, 1703 - 1705 (2010): "c-di-AMP Secreted by Intracellular Listeria monocytogenes Activates a Host Type I Interferon Response"

Rao, F.; See, R.Y.; Zhang, D.; Toh, D.C.; Ji, Q.; Liang, Z.-X., *J. Biol. Chem.*, **285**, 473 - 482 (2010): "YybT is a Signaling Protein that Contains a Cyclic Dinucleotide Phosphodiesterase Domain and a GGDEF Domain with ATPase Activity"

Römling, U., Sci. Signal., 1(33), pe39 (2008): "Great Times for Small Molecules: c-di-AMP, a Second Messenger Candidate in Bacteria and Archaea"

Witte, G.; Hartung, S.; Büttner, K.; Hopfner, K.-P., *Mol. Cell*, **30**, 167 - 178 (2008): "Structural Biochemistry of a Bacterial Checkpoint Protein Reveals Diadenylate Cyclase Activity Regulated by DNA Recombination Intermediates"

Simm, R.; Lusch, A.; Kader, A.; Andersson, M.; Römling, U., *J. Bacteriol.*, **189**, 3613 - 3623 (2007): "Role of EAL-containing Proteins in Multicellular Behavior of Salmonella enterica Serovar Typhimurium"