PRODUCT INFORMATION

3’3’-cGAMP Affinity Sorbent

Item No. 400572

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Overview

Contents: This vial contains 1.2 or 12 ml of 3’3’-cGAMP affinity sorbent (mouse anti-3’3’-cGAMP antibody covalently bound to Sepharose 4B) supplied in Affinity Column Buffer (1X).
Synonyms: Adenosine-Guanosine 3’,3’-cyclic monophosphate, 3’,3’-Cyclic GMP-AMP, 3’3’-cGAMP Affinity Beads, 3’3’-cGAMP Affinity Resins, c-GpAp
Storage: 4°C
Stability: ≥18 months
Conditions: Single use only.

Description

One milliliter of sorbent is capable of binding approximately 50 ng of 3’3’-cGAMP. The sorbent can be used for purification, concentration, or removal of 3’3’-cGAMP from biological samples.

Preparations

Sample Preparation

All samples should be at neutral pH. Centrifuge samples briefly to remove particulates and precipitates and dilute 1:4 with Affinity Column Buffer (1X) (see below) before purification. It is recommended to use 1 ml of diluted sample per 200 µl of sorbent slurry. When using larger sample volumes or higher expected sample concentrations of 3’3’-cGAMP, the amount of sorbent and incubation time required for binding the 3’3’-cGAMP may need to be increased accordingly.

Buffer Preparation

Affinity Column Buffer (azide free) (5X) (Item No. 400690) is available for purchase. Dilute 25 ml of the concentrated buffer with 100 ml of ultrapure water to make column buffer (Affinity Column Buffer (1X)). Mix well before use.

Protocol for Purification or Removal of 3’3’-cGAMP

1. Gently invert the 3’3’-cGAMP Affinity Sorbent (Item No. 400572) to make a homogenous slurry. Transfer the necessary amount of sorbent to a microcentrifuge or similar tube. This volume will be designated as the sorbent volume (SV) and will serve as the reference volume for subsequent steps. Briefly centrifuge at 100 x g with gentle braking to sediment the sorbent slurry. Remove and discard the storage buffer.
2. Wash the sorbent by adding two SVs of column buffer to the sorbent, mix gently, and centrifuge, then remove and discard the supernatant. Repeat the wash process with another two SVs of column buffer.
3. Add the diluted sample to the washed sorbent and mix the contents together to make a homogenous slurry.
4. Mix gently for 60 seconds.
5. Briefly centrifuge the sample at 400 x g to sediment the sorbent.

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6. Carefully remove the supernatant with a pipette or by decanting. Care must be taken to retain all the sorbent as it contains bound 3'3'-cGAMP. Discard the supernatant. *Optional: the supernatant can be kept and tested by LC-MS or an immunoassay, such as Cayman's 3'3'-cGAMP ELISA Kit (Item No. 502130) to confirm depletion of 3'3'-cGAMP.*
7. Wash the sorbent with two SVs of column buffer, centrifuge, and carefully remove and discard the supernatant solution. Repeat the wash.
8. Wash the sorbent with two SVs of ultrapure water, centrifuge, and carefully remove and discard the supernatant solution. Repeat the wash.
9. Elute by resuspending the sorbent pellet in two SVs of ice-cold methanol. Vortex briefly.
10. Centrifuge to sediment the sorbent and carefully transfer the methanol containing 3'3'-cGAMP to a clean tube. Repeat this elution a second time combining the elution into one tube.
11. For mass spectroscopy, eluates may be analyzed directly or further processed according to established LC-MS preparation protocols. For immunoassay-based detection (*e.g.*, Cayman's 3'3'-cGAMP ELISA Kit), evaporate the methanol and resuspend the purified sample in an appropriate buffer.

    NOTE: If immediate analysis is not possible, evaporate the methanol to dryness using a vacuum centrifuge or under a stream of dry nitrogen. Resuspend the sample in ultrapure water and store at -80°C. Samples are stable under these conditions for up to six months.

Sample Data

L. plantarum lysate prepared in B-PER™ was spiked with the indicated amounts of 3'3'-cGAMP (Item No. 17966), purified according to the protocol outlined above, reconstituted in 1 ml of ultrapure water, and analyzed by LC-MS/MS.

Spiked 3'3'-cGAMP (ng)	Measured 3'3'-cGAMP (ng)	% Recovery
50.0	66.8	132.5
25.0	26.7	104.7
12.5	16.6	128.6
6.25	7.92	117.8
3.13	4.78	135.1
1.56	2.95	152.9
0	0.557	

Table 1. Spike and recovery in *L. plantarum* lysate measured by LC-MS/MS

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