PRODUCT INFORMATION

Cyclic di-AMP Affinity Sorbent

Item No. 400573

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Overview

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

Description

One milliliter of sorbent is capable of binding approximately 70 ng of cyclic di-AMP. The sorbent can be used for purification, concentration, or removal of cyclic di-AMP 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. When using larger sample volumes or higher expected sample concentrations of cyclic di-AMP, the amount of sorbent and the incubation time required for binding the cyclic di-AMP 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 cyclic di-AMP

1. Gently invert the Cyclic di-AMP Affinity Sorbent (Item No. 400573) to make a homogeneous 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, centrifuge, and 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 homogeneous slurry.
4. Mix gently for 60 minutes.
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 cyclic di-AMP. Discard the supernatant. *Optional: the supernatant can be kept and tested by LC-MS or immunoassay (Cyclic di-AMP ELISA Kit; Item No. 501960) to confirm depletion of cyclic di-AMP.*
7. Wash the sorbent with two SVs of column buffer, centrifuge, and carefully remove and discard the supernatant. Repeat the wash.
8. Wash the sorbent with two SVs of ultrapure water, centrifuge, and carefully remove and discard the supernatant. 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 cyclic di-AMP to a clean tube. Repeat this elution a second time combining the elution into one tube.
11. For mass spectrometry, eluates may be analyzed directly or further processed according to established LC-MS preparation protocols. For immunoassay-based detection (*e.g.*, Cayman's Cyclic di-AMP ELISA Kit), evaporate the methanol and resuspend the purified sample in the ELISA 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.

Performance Characteristics

E. coli lysate prepared in B-PER™ was spiked with the indicated amount of cyclic di-AMP (Item No. 17753), purified according to the protocol outline in this kit booklet, reconstituted in 1 ml of ultrapure water, and analyzed by LC-MS/MS.

Spiked cyclic di-AMP (ng)	Measured cyclic di-AMP (ng)	% Recovery
50.0	47.3	94.5
37.5	30.3	80.9
25.0	20.6	82.5
12.5	10.6	84.5
6.25	5.34	85.5
3.13	2.56	82.0
1.57	1.42	90.9
0.78	0.709	90.9
0.39	0.362	92.8
0.20	0.209	107
0	*	

Table 1. Spike and recovery in *E. coli* lysate measured by LC-MS/MS
**Below lower limit of quantification*

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