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## Cyclic di-AMP Affinity Purification Kit

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Item No. 502790

[www.caymanchem.com](http://www.caymanchem.com)

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## TABLE OF CONTENTS

<b>GENERAL INFORMATION</b>	3	Materials Supplied
	4	Safety Data
	4	Precautions
	4	If You Have Problems
	5	Storage and Stability
	5	Materials Needed But Not Supplied
<b>INTRODUCTION</b>	6	About This Kit
<b>ASSAY PROTOCOL</b>	7	Reagent Preparation
	7	Sample Preparation
	8	Purification Protocol
<b>ANALYSIS</b>	9	Performance Characteristics
<b>RESOURCES</b>	10	Notes
	11	Warranty and Limitation of Remedy

## GENERAL INFORMATION

### Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
400690	Affinity Column Buffer (azide free) (5X)	1 vial/25 ml	RT
400573	Cyclic di-AMP Affinity Sorbent	1 vial/12 ml	4°C
401115	Mini Chromatography Column	10 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



**WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.**

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this purification.**

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Cyclic di-AMP Affinity Purification Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

## If You Have Problems

### Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A source of ultrapure water, with a resistivity of 18.2 M $\Omega$ -cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
2. Ice-cold methanol

## About This Kit

Cyclic di-AMP Affinity Purification Kit can be used for purification, concentration, or removal of cyclic di-AMP from biological samples. Cyclic di-AMP Affinity Sorbent (Item No. 400573) exhibits a binding capacity of approximately 70 ng/ml for cyclic di-AMP.

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## Reagent Preparation

### 1. Prepare Affinity Column Buffer (2X)

Dilute 10 ml of Affinity Column Buffer (azide free) (5X) (Item No. 400690) with 15 ml of ultrapure water to make Affinity Column Buffer (2X). Mix well before use. Affinity Column Buffer (2X) is used to wash the column **AFTER** the sample has been loaded. The diluted buffer is stable at room temperature for six months.

### 2. Prepare Affinity Column Buffer (1X)

Dilute 10 ml of Affinity Column Buffer (azide free) (5X) (Item No. 400690) with 40 ml of ultrapure water to make Affinity Column Buffer (1X). Mix well before use. Affinity Column Buffer (1X) is used to wash the column **BEFORE** loading the sample. The diluted buffer is stable at room temperature for six months.

### 3. Affinity Column Preparation

Gently invert the Cyclic di-AMP Affinity Sorbent (Item No. 400573) to make a homogeneous slurry. Add 1 ml of slurry to each Mini Chromatography Column (Item No. 401115), cap, and keep upright. Allow the slurry to settle before purification. Columns can be stored at 4°C for six months following addition of sorbent. *NOTE: These columns are intended to be a single-use device (one sample per column).*

## Sample Preparation

This kit has been validated in *E.coli* lysates. Centrifuge all samples briefly to pellet sediment and ensure they are free of particulates and precipitates. Samples should be at neutral pH and diluted 1:4 with Affinity Column Buffer (1X) prior to purification. Note that the purification protocol has been optimized for a final sample volume of 2 ml.

## Purification Protocol

1. Remove the bottom plug and allow the storage solution to pass through the sorbent. Wash the column with 2 ml of Affinity Column Buffer (1X). Repeat wash and discard the flow-through. *NOTE: Make sure the flow through the column has completely stopped before adding the next solution/proceeding to the next step.*
2. Load 2 ml of sample on the column, allowing it to flow by gravity.
3. Wash with 2 ml of Affinity Column Buffer (2X), followed by 2 ml of ultrapure water. Allow all the water to pass through the sorbent. Discard both of the washes. *Optional: the flow-through can be kept and assayed to determine purification efficiency.*
4. Place a test tube capable of holding at least 5 ml under the column. Elute the cyclic di-AMP from the column by adding 1 ml of ice-cold methanol and allowing it to pass through the sorbent completely. Repeat with an additional 1 ml of ice-cold methanol.
5. For mass spectrometry, eluates in methanol can be analyzed directly or processed according to established LC-MS sample preparation procedures. For immunoassay-based detection (e.g., Cayman's Cyclic di-AMP ELISA Kit (Item No. 501960)), evaporate the methanol and reconstitute the purified sample in the ELISA buffer.

*NOTE: If immediate analysis is not possible, evaporate the methanol 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.*

## ANALYSIS

### 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, the methanol was evaporated and the sample was 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

### Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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