



3'3'-cGAMP Affinity Purification Kit

Item No. 502864

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	10 ea Quantity/Size	Storage Temperature
400690	Affinity Column Buffer (azide free) (5X)	1 vial/25 ml	RT
400572	3'3'-cGAMP Affinity Sorbent	1 vial/12 ml	4°C
401115	Mini Chromatography Column	10 columns	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 assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's 3'3'-cGAMP 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 Ω -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

INTRODUCTION

About this Kit

The 3'3'-cGAMP Affinity Purification Kit can be used for purification, concentration, or removal of 3'3'-cGAMP from biological samples. The 3'3'-cGAMP Affinity Sorbent (Item No. 400572) exhibits a binding capacity of approximately 50 ng/ml for 3'3'-cGAMP.

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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 will be 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 will be stable at room temperature for six months.

3. Affinity Column Preparation

Gently invert the 3'3'-cGAMP Affinity Sorbent (Item No. 400572) 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 devices (one sample per column).*

Sample Preparation

This kit has been validated in *E. coli* and *L. plantarum* lysates. Centrifuge all samples briefly to pellet sediment and ensure they are free of all 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 from a Mini Chromatography Column and allow the storage solution to pass through the sorbent. Wash the column with 2 ml of Affinity Column Buffer (1X). Repeat the wash and discard the flow through. *NOTE: Make sure the flow through has completely stopped before adding the next solution/proceeding to the next step.*
2. Apply the sample to the column allowing it to flow through by gravity.
3. Wash the column with 2 ml of Affinity Column Buffer (2X), followed by 2 ml ultrapure water. Allow all the water to pass through the sorbent. Discard both of these 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 3'3'-cGAMP 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 immunoassay-based detection (e.g. Cayman's 3'3'-cGAMP ELISA Kit (Item No 502130)), evaporate the methanol and reconstitute the purified sample in Immunoassay Buffer C. For mass spectrometry, eluates in methanol can be analyzed directly or processed according to established LC-MS sample preparation procedures. *NOTE: If the analysis cannot be performed immediately, resuspend the dried sample in 1 ml ultrapure water and store at -80°C, where it will be stable for six months.*

ANALYSIS

Performance Characteristics

Spike and Recovery

L. plantarum prepared in B-PER™ Bacterial Protein Extraction Reagent was spiked with the indicated amount of 3'3'-cGAMP (Item No 17966) and purified according to the Purification Protocol (see page 8). The methanol was then evaporated and the sample reconstituted with 1 ml of ultrapure water and analyzed by LC-MS/MS.

Spiked 3'3'-cGAMP Concentration (ng/ml)	Measured 3'3'-cGAMP Concentration (ng/ml)	% Recovery
0	0.557	--
1.56	2.95	153.4
3.13	4.78	134.9
6.25	7.92	117.8
12.5	16.6	128.3
25	26.7	104.6
50	66.8	132.5

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

Warranty and Limitation of Remedy

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