APPLICATION NOTE

Cyclic di-GMP assay confirms antibacterial lowering of c-di-GMP levels

A powerful tool for evaluating virulence & biofilm formation

Introduction

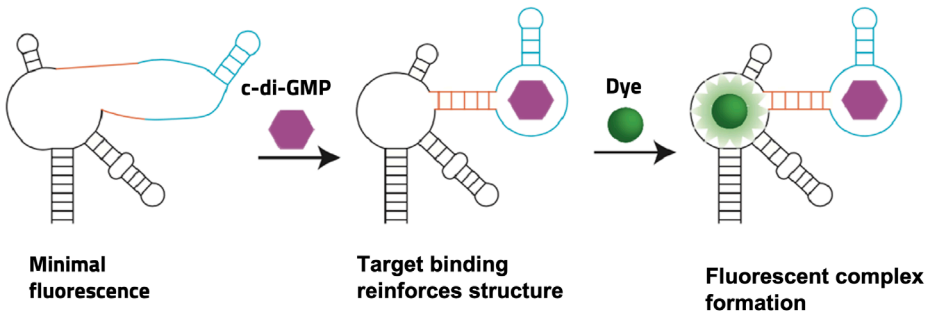
Cyclic-di-guanosine monophosphate (cyclic di-GMP or c-di-GMP) is a crucial second messenger that regulates key bacterial biological processes. Intracellular c-di-GMP levels play a pivotal role in controlling bacterial pathogenicity, such as biofilm formation, dispersal, motility, and virulence. To advance the study of bacteria biology and the discovery of new antibacterial drugs, robust and easy-to-use tools are essential for accurate determinations of intracellular c-di-GMP levels.

Features

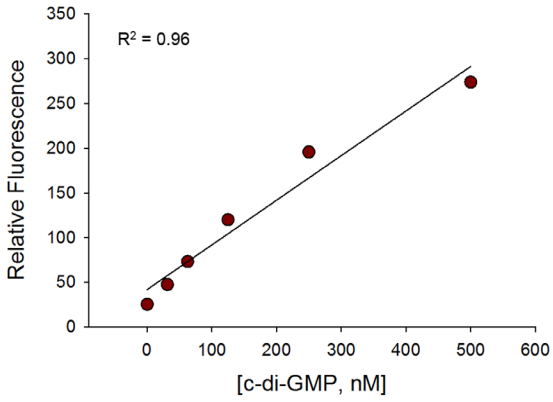
- Sensitive and specific detection of c-di-GMP concentrations
- Compatible with both biochemical and cell samples
- Suitable for high-throughput screening of novel antibacterial compounds

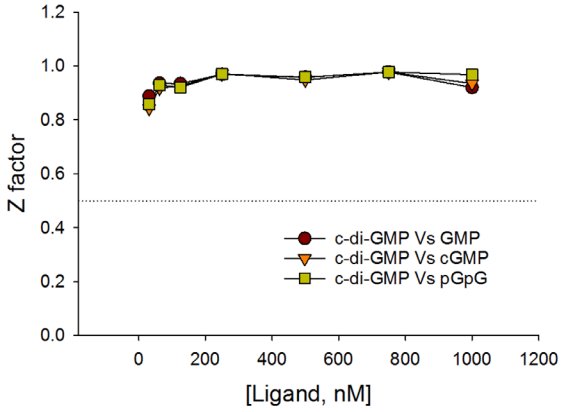
The Assay

The Cyclic-di-GMP assay utilizes riboswitch-based ligand detection in a simple, specific format to enable high-throughput measurement of c-di-GMP levels in cells. This homogenous assay can be applied to detect c-di-GMP in biochemical or enzymatic reactions that produce c-di-GMP, as well as cell-based applications to monitor intracellular c-di-GMP changes.

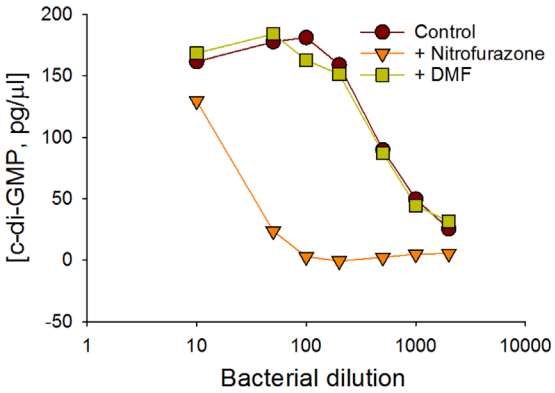


Components	Notes
Cyclic-di-GMP assay	Cyclic-di-GMP Assay (Lucerna, Cat. #200-100)
c-di-GMP standards	Included in the Cyclic-di-GMP Assay
Bacterial cell samples	Bacterial cultures or lysates
Assay plates	96- and 384-well black assay plates suitable for fluorescence reading
Plate readers	Fluorescence plate reader with FITC filter set or capable of excitation at 482nm and emission at 505nm
Liquid handling devices	Devices that accurately dispense low microliter volumes
Water and plasticware	Everything should be nuclease-free





The Cyclic di-GMP assay has a broad dynamic range and achieves excellent Z factor (Z'>0.9) even in the presence of 500 - 1,000-fold excess of counter ligands, such as GMP, pGpG, and c-AMP-GMP.



E. coli cells expressing wild type WspR, a diguanylate cyclase from *P. aeruginosa*, were treated with nitrofurazone, dimethyl formamide (DMF), or left untreated (control). Drug-dependent reductions in c-di-GMP levels were detected across varying bacterial cell densities. Intracellular c-di-GMP concentrations were measured directly from cells grown in 96-well plates, without any pelleting, washes, lysis, or organic extraction steps.

Lucerna is utilizing Spinach™ technology to:

1. **Provide plug-and-play RNA imaging and detection products,**
2. **Develop HTS platforms to enable new RNA-targeted drug discovery**
3. **Create fluorescent sensors for industrial production applications.**

This product was cited in:

1. Zhao *et al.* Mol Microbiol, 2022. PMID: 35072315
2. Piazza *et al.* mBio, 2022. PMID: 36374078
3. Leighton *et al.* Front Microbiol, 2023. PMID: 37854331
4. Sun *et al.* Microbiol Res, 2024. PMID: 37922698
5. Guo *et al.* Microbiol Res, 2024. PMID: 39116779

Scan to learn more.

LUCERNA™

760 Parkside Ave.
Brooklyn, NY 11226

contact@lucernatechnologies.com
www.lucernatechnologies.com