

## MycoLight™ Cyclic-di-GMP Red Sensor

Catalog number: 17103

Unit size: 1 mg

Component	Storage	Amount (Cat No. 17103)
MycoLight™ Cyclic-di-GMP Red Sensor	Freeze (< -15 °C), Minimize light exposure	1 mg

### OVERVIEW

The MycoLight™ Cyclic-di-GMP Red Sensor is developed to fluorometrically detect cyclic-di-GMP (c-di-GMP). The sensor generates a red fluorescent signal upon selectively binding to c-di-GMP. The second messenger 3',5'-cyclic-di-guanosine monophosphate (c-di-GMP) is ubiquitous in bacteria and regulates a wide range of bacterial functions, including biofilm formation, which can lead to chronic infections and antibiotic resistance. C-di-GMP exists in both monomer and dimer forms. Currently, there is a lack of sensitive probes for detecting c-di-GMP. The MycoLight™ Cyclic-di-GMP Red Sensor addresses this unmet need, offering high sensitivity and selectivity towards the c-di-GMP structure with minimal interference from other secondary messengers like cyclic AMP. Its larger Stokes shift provides improved signals and allows for multiplexing with other fluorophores in imaging applications without spectral overlap.

### AT A GLANCE

1. Grow bacteria in a desired growth medium.
2. Add MycoLight™ Cyclic-di-GMP Red Sensor working solution.
3. Incubate cells for 10 to 30 minutes at RT.
4. Monitor fluorescence in Cy5 or violet filter set

**Note:** Before first use, thaw MycoLight™ Cyclic-di-GMP Red Sensor at room temperature and centrifuge briefly to collect the dried pellet.

### KEY PARAMETERS

#### Instrument: Fluorescence microscopy

Excitation: Violet or Cy5 filter

Emission: Violet or Cy5 filter

Recommended plate: Black-clear bottom plate

#### Instrument: Fluorescence microplate readers

Excitation: 450 or 490 nm

Emission: 650 nm

Cutoff = 590 nm

Recommended plate: Black solid plate (for solution based assay)

### PREPARATION OF STOCK SOLUTIONS

Prepare 10 to 20 mM stock solution in DMSO. e.g. Add 210 µL of DMSO into MycoLight™ Cyclic-di-GMP Red Sensor vial to make 10 mM stock solution.

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles*

### PREPARATION OF WORKING SOLUTION

Prepare 10 to 20 µM MycoLight™ Cyclic-di-GMP Red Sensor working solution. For example, add 2 µL of 10 mM MycoLight™ Cyclic-di-GMP Red Sensor stock solution into 998 µL of 1X PBS buffer (for live bacteria staining) or 10 mM Tris-HCl, pH 7.5 containing 60 mM KCl (for solution based assay) to make 20 µM MycoLight™ Cyclic-di-GMP Red Sensor working solution.

**Note:** Protect the working solution from light by covering it with foil or placing it in the dark.

**Note:** For best results, this solution should be used within a few hours of its preparation.

### SAMPLE EXPERIMENTAL PROTOCOL

The following protocols only provides a guideline and should be modified according to your specific needs.

#### Live bacteria staining with MycoLight™ Cyclic-di-GMP Red Sensor:

1. Grow bacteria in growth medium as desired.
2. Treat cells as desired to induce Cyclic-di-GMP.
3. Centrifuge bacterial cells at 10,000 RPM for 10 mins to remove the growth medium and wash bacterial cells with 1X PBS.
4. Add 100 µL of MycoLight™ Cyclic-di-GMP Red Sensor working solution to cells.
5. Incubate cells for 10 to 30 minutes at RT, protected from light.
6. Monitor fluorescence with fluorescence microscope using Violet or Cy5 filter set (best Ex/Em = 490/650 nm) .

**Note:** The concentration and incubation time of MycoLight™ Cyclic-di-GMP Red Sensor used varies with different bacterial strains, one will need test with different concentrations to get the optimal dose.

#### Solution assay with MycoLight™ Cyclic-di-GMP Red Sensor:

##### Table 1. Layout of Cyclic-di-GMP standards and test samples in a solid black 96-well microplate.

cGMP = Cyclic-di-GMP Standards (cGMAP1 - cGMP7, 800 to 12.5 µM, 2X dilutions); BL=Blank Control; TS=Test Samples

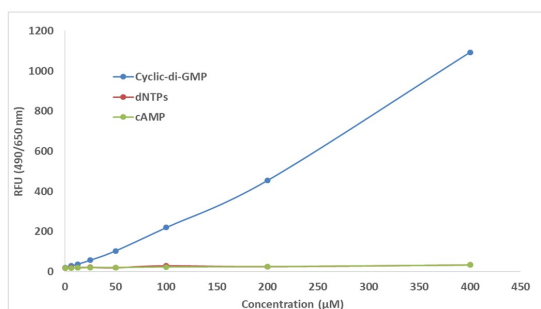
BL	BL	TS	TS
cGMAP1	cGMAP1	---	---
cGMAP2	cGMAP2	---	---
cGMAP3	cGMAP3	---	---
cGMAP4	cGMAP4	---	---
cGMAP5	cGMAP5	---	---
cGMAP6	cGMAP6	---	---
cGMAP7	cGMAP7	---	---

**Table 2. Reagent composition for each well.**

Well	Volume	Reagent
cGMP1-cGMP7	50 $\mu$ L	Serial dilutions (800 to 12.5 $\mu$ M)
BL	50 $\mu$ L	Tris Buffer
TS	50 $\mu$ L	Sample

1. Prepare cyclic-di-GMP standards (cGMP, not provided), blank controls (BL), and test samples (TS) according to the layout provided in tables 1 and 2. For a 384-well plate, use 25  $\mu$ L of reagent per well instead of 50  $\mu$ L.
2. Add 50  $\mu$ L of MycoLight™ Cyclic-di-GMP Red Sensor working solution to each well of cyclic-di-GMP standards, blank control, and test samples to make the assay volume of 100  $\mu$ L/well. For a 384-well plate, add 25  $\mu$ L into each well instead, for a total volume of 50  $\mu$ L/well.
3. Incubate the reaction at room temperature for 5 to 10 minutes, protected from light.
4. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 490/650 nm (cut off at 590 nm).

**EXAMPLE DATA ANALYSIS AND FIGURES**



**Figure 1.** The fluorescence intensity of MycoLight™ Cyclic-di-GMP Red Sensor was measured at 650 nm (excitation at 490 nm) in the presence of Cyclic-di-GMP (Blue), dNTPs (Red) or cyclic-AMP (Green) ranging from 400 to 6.125  $\mu$ M in 10 mM Tris-HCl, pH 7.5 containing 60 mM KCl buffer. MycoLight™ Cyclic-di-GMP Red Sensor is specific to Cyclic-di-GMP and has no response for dNTPs and cyclic-AMP.

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