

Luciferase Mycoplasma Detection Kit

Catalogue No.: abx298016

Luciferase Mycoplasma Detection Kit exploits the activity of certain mycoplasma metabolic enzymes that are rich in most kinds of mycoplasma. The enzymes react with the substrate catalyzing the conversion of ADP to ATP. By measuring the level of ATP in a sample with a luciferase assay both before and after the addition of Substrate, viable mycoplasma contamination can be detected. This assay provides a fast, simple and sensitive method to detect mycoplasma contamination in cell cultures and cell culture materials. As this assay can only detect bioactive mycoplasma, the results will be more accurate than that of the PCR assay.

Contents:

Component	25 reactions	50 reactions
Reagent (Lyophilized)	2 vials	4 vials
Substrate (Lyophilized)	2 vials	4 vials
Positive Control	250 µl	500 µl
Mycoplasma-free Water (1.5 ml)	2 vials	4 vials

Target: Luciferase Mycoplasma Detection Kit

Reconstitution: Reconstitute the Reagent with 700 µl Mycoplasma-free Water. Reconstitute the Substrate with 700 µl Mycoplasma-free Water. After reconstitution, the solutions should be used immediately in the assay or stored according the storage recommendation.

Storage: Store at -20°C in the dark for up to one year. After reconstitution, the Reagent and Substrate can be stored at 2-8°C for up to one week, -20°C for up to one month or -70°C for up to 6 months. Avoid repeated/freeze thaw cycles.

Note: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.
This product is shipped with dry ice.

Directions for use: Cell Culture Collection

Culture the cells for at least 24 hours and then collect the cell culture medium. Centrifuge at $400 \times g$ for 3 minutes. The supernatant should be tested immediately or be stored at 2-8 °C for up to one week. Avoid freezing and thawing the collected medium. For optimal assay performance, cell confluency should reach 80% or higher.

Assay Procedure

1. Carry out the assay away from bright light.
2. Equilibrate the reconstituted Reagent, Substrate, Positive Control, Negative Control (PBS, fresh medium or sterile water) and cell culture medium supernatant to room temperature.
3. Add 50 µl Reagent, 50 µl cell culture medium supernatant and 50 µl of the positive and negative controls to a 1.5 ml tube or 96-well plate. It is recommended to set up one positive control per assay. Mix samples gently with a pipette and avoid generating foam or large bubbles. Incubate at room temperature for 5-10 minutes.
4. Place the tube or 96-well plate in a luminometer to measure the luminescent signal value at 560 nm (Reading A).
5. Add 50 µl of Substrate to the tube or 96-well plate. Mix samples gently with a pipette and avoid generating foam or large bubbles. Incubate at room temperature for 10-15 minutes.
6. Place the tube or 96-well plate in a luminometer to measure the luminescent signal value at 560 nm (Reading B).
7. Calculate the ratio of Reading B to Reading A:

Sample to be detected:

- $B/A \geq 1$: Samples have Mycoplasma contamination.
- $0.8 < B/A < 1$: Quarantine samples and retest after 24-48 hours. If the B/A ratio remains between 0.8 and 1 with no significant increase, the sample can be considered negative for mycoplasma.
- $B/A \leq 0.8$: Negative for Mycoplasma.

Positive Control:

- $B/A \geq 5$: The results are valid.
- $B/A < 5$: Results are invalid.