AllDetect Luciferase Mycoplasma Detection Kit

Please read the datasheet carefully prior to use.
Cat. No. ABTGDT301-01
Version number: Version 2.0
Storage: At -20°C in dark for one year. Reconstituted MycoDetect Reagent and MycoDetect Substrate can be stored at -70°C for six months, at -20°C for one month, or at 2-8°C for one week.

Description

This product is designed using an enzyme unique to mycoplasma. This enzyme degrades MycoDetect Substrate and converts ADP to ATP. Luciferase catalyzes oxidation of luciferin to produce bioluminescence in the presence of ATP. Cell cultures can be characterized for the presence of mycoplasma contamination by detecting bioluminescence. As this assay can only detect bioactive mycoplasma, the results will be more accurate than that of the PCR assay. The method has high sensitivity, simple operation and time saving.

Kit Contents

Component	ABTGDT301-01	ABTGDT301-02
MycoDetect Reagent (lyophilized)	2 Vials	4 Vials
MycoDetect Substrate (lyophilized)	2 Vials	4 Vials
AllDetect Luciferase Mycoplasma Detection Positive Control	250 µl	500 µl
MycoFree Water	2×1.5 ml	4×1.5 ml

Procedures

1. Reagent Preparation

(1) Add 700 µl of MycoFree Water to fully dissolve lyophilized MycoDetect Reagent and MycoDetect Substrate, respectively.
Note: To ensure accurate results, we suggest that the MycoDetect Reagent and MycoDetect Substrate are used immediately after reconstitution. If it cannot be used up once, the reconstituted MycoDetect Reagent and MycoDetect Substrate should be stored at -20°C.

(2) To avoid repeated freeze-thaw, the positive control agent AllDetectLuciferase Mycoplasma Detection Positive Control should be aliquoted and stored at -20°C.

2. Collect Cell Culture

Culture the cells for at least 24 hours and then collect the cell culture medium, centrifuge at 400×g for 3 minutes. The supernatant should be detected immediately or be stored at 2-8°C for no more than one week. Avoid freezing and thawing the collected cell culture medium.
Note: For optimal assay performance, cell confluency should reach 80% or higher.

3. Assay (away from light)

(1) Equilibrate the dissolved MycoDetect Reagent, MycoDetect Substrate, positive control reagent and cell culture medium supernatant to room temperature.

(2) Add 50 µl cell culture medium supernatant, positive control and negative control (sterile water, PBS, or fresh culture medium) to a 1.5 ml tube or 96-well plate. It is recommended to set up one positive control per assay.

(3) Add 50 µl MycoDetect Reagent, mix well with a pipette and incubate at room temperature for 5-10 minutes.
Note: Mix the samples gently with a pipette and be careful not to generate any large bubbles. Small bubbles on the edge of the tube or well have no influence on the results.

(4) Place the tube or 96-well plate in luminometer to measure the luminescent signal value (Reading A).

(5) Add 50 µl of MycoDetect Substrate to the reaction system in step (3). Mix well with a pipette and incubate at room temperature for 10-15 minutes.
Note: Mix the samples gently with a pipette and be careful not to generate any large bubbles. Small bubbles on the edge of the tube or well have no influence on the results.

(6) Place the tube or 96-well plate in luminometer to measure the luminescent signal value (Reading B).

(7) Data interpretation:

Positive control: if B/A ≥ 5, the results of this experiment are credible; If B/A < 5, the kit activity is reduced and the results cannot be credible.
Sample to be detected: if B/A≥1, there is mycoplasma contamination in cell culture; If B/A ≤ 0.8, there is no mycoplasma contamination in the cell culture; if B/A is between 0.8-1, it is recommended to continue culture the cells for 24-48 hours to detect mycoplasma contamination again, and if B/A is still between 0.8-1, the cell culture is mycoplasma negative.