TransDetect® Luciferase Mycoplasma Detection Kit

Please read the instructions carefully before use

Cat. No. FM301

Storage

At -20℃ in dark for 1 year. Reconstituted MycoDetect Reagent and MycoDetect Substrate can be stored at -70℃ for six months, at -20℃ for one month, or at 2-8℃ for one week.

Description

TransDetect® 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 MycoDetecct 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.

Kit Contents

Component	FM301-01 (25 rxns)	FM301-02 (50 rxns)
MycoDetect Reagent (lyophilized)	2 Vials	4 Vials
MycoDetect Substrate (lyophilized)	2 Vials	4 Vials
MycoFree Water	2 ×1.5 ml	4×1.5 ml

Procedures

• Component Reconstitution

Reconstitute lyophilized one vial of MycoDetect Reagent and one vial of MycoDetect Substrate by adding 700 µl MycoFree Water respectively. Wait until rehydration completes.

Note: To obtain the best results, we suggest that the MycoDetect Reagent and MycoDetect Substrate are used immediately after reconstitution. The reconstituted MycoDetect Reagent and MycoDetect Substrate should be stored following the storage recommendation.

• 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 tested immediately or be stored at 2-8°C for no more than one week. Avoid freezing and thawing the collected medium.

Note: For optimal assay performance, cell confluency should reach 80% or higher.

• Assay (avoid bright lighting)

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

(2) Add 50 µl MycoDetect Reagent and 50 µl cell culture medium supernatant to a 1.5 ml tube or 96-well plate, 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.

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

(4) Add 50 µl of MycoDetect Substrate to the tube or 96-well plate 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.

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

(6) Data interpretation: The ratio of Reading B to Reading A is used to determine whether a cell culture is contaminated by mycoplasma.

Ratio	Interpretation
B/A ≥1	Mycoplasma contamination
0.8＜B/A ＜1	Borderline※
B/A ≤0.8	Negative for Mycoplasma

※If the B/A ratio is between 0.8 and 1, samples should be retested after another 24-48 hours culture in quarantine. If the B/A ratio remain between 0.8 and 1 with no significant increase, the sample is mycoplasma negative.

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