

Myco-Sniff-Rapid™

Mycoplasma Luciferase Detection Kit

DESCRIPTION

Myco-Sniff-Rapid™ Mycoplasma Luciferase Detection Kit offers a quick (20 mins), simple and sensitive method for detecting mycoplasma contamination in cell cultures. This innovative kit utilizes the activity of mycoplasma metabolic enzymes from 209 mycoplasma species, including those commonly found contaminating cell cultures. Since this enzyme is not present in eukaryotic cells, the test is specific for mycoplasma. In the presence of mycoplasma, the enzymes react with the substrate to catalyze the conversion of ADP to ATP. Mycoplasma contamination can be detected by measuring the level of ATP in a sample before and after the addition of Myco-Sniff-Rapid substrate via a luciferase assay.

BENEFITS

- Rapid and highly sensitive results in 20 minutes
- Specific for detecting all common mycoplasma contaminant species
- No interference with cell culture media components
- Simple operation—add reagents directly to the culture supernatant

COMPONENTS

Component	093050401 (25 tests)	093050402 (50 tests)
Myco-Sniff-Rapid Reagent (lyophilized)	2 vials	4 vials
Myco-Sniff-Rapid Substrate (lyophilized)	2 vials	4 vials
Mycoplasma Detection Positive Control	250 µL	500 µL
Myco-Free Water	2 x 1.5 mL	4 x 1.5 mL

STORAGE

- Store kit at -20 °C in the dark for 1 year.
- Reconstituted Myco-Sniff-Rapid Reagent and Substrate work best when used immediately, but can be stored at -80 °C for up to six months, at -20 °C for up to one month, or at 2–8 °C for up to one week.
- Aliquot and store the Mycoplasma Detection Positive Control at -20 °C to avoid repeated freeze-thaw cycles.

MATERIALS REQUIRED, BUT NOT SUPPLIED

- 96-well plate
- Plate reader with luminescence measurement function
- Pipette
- Pipette tips
- Centrifuge

PROCEDURE

COMPONENT RECONSTITUTION:

Reconstitute one vial of lyophilized Myco-Sniff-Rapid Reagent and one vial of Myco-Sniff-Rapid Substrate by adding 700 µL Myco-Free water to each. Wait until rehydration is completed.

COLLECT CELL CULTURE:

Culture the cells for at least 24 hours, collect the cell culture medium and centrifuge at 400 × g for 3 minutes. The supernatant should be tested immediately or 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 (PROTECT FROM LIGHT):

- 1 Equilibrate Myco-Sniff-Rapid Reagent, Myco-Sniff-Rapid Substrate, Mycoplasma Detection Positive Control reagent, and cell culture medium supernatant solutions to room temperature.
- 2 Add 50 µL cell culture medium supernatant, positive control, and negative control (sterile water, PBS, or fresh culture medium) respectively to a white 96-well plate. It is recommended to set up one positive control per assay.
- 3 Add 50 µL Myco-Sniff-Rapid Reagent to the above solutions in the 96-well plate. Mix by carefully pipetting up and down, then 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 well should have no influence on the results.

- 4 Place the 96-well plate in a plate reader and select Luminescence Measurement, emission 562 nm (Reading A).
- 5 Add 50 µL of Myco-Sniff-Rapid 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 well should have no influence on the results.

- 6 Place the 96-well plate in a plate reader and select Luminescence Measurement, emission 562 nm (Reading B).
- 7 Data interpretation: The ratio of Reading B to Reading A is used to determine whether a cell culture is contaminated by mycoplasma.

Ratio (Reading B/Reading A)	Results
$B/A \geq 1$	Mycoplasma contamination
$B/A \leq 0.8$	Negative for mycoplasma
$0.8 < B/A < 1$	Samples should be retested after another 24–48 hours culture in quarantine. If the B/A ratio remains between 0.8 and 1 with no significant increase, the sample is negative for mycoplasma.

POSITIVE CONTROL: if $B/A \geq 5$, the procedure worked as expected and sample results are valid; If $B/A < 5$, kit activity has been reduced or the procedure was conducted improperly and results may not be valid.



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