

## Introduction

Mycoplasma Luminescent Detection Kit allows for quick and reliable screening of cell cultures for contamination with mycoplasmas. The assay utilizes the activity of mycoplasma-specific enzymes and detects through the luminescence reaction catalyzed by ATP-dependent luciferase. The entire detection process has only two steps and only takes about 15 minutes. The first step is to add mycoplasma detection reagent A to the sample and perform the test after 5 minutes. The reading value is A. The second step is to add mycoplasma detection reagent B and perform the test after 10 minutes. The reading value is B. If the sample is contaminated by mycoplasma, its unique enzyme can convert the ADP into ATP. By measuring the level of ATP in a sample both before and after the addition of the mycoplasma detection reagent B, a ratio can be obtained which is indicative of the presence or absence of mycoplasma.

## Package Information

Components	M0133
Mycoplasma Detection Reagent A, lyophilized	1 ml
Mycoplasma Detection Reagent B, lyophilized	1 ml
Mycoplasma Assay Buffer	10 ml

## Storage

Mycoplasma Detection Reagent A & B at -20°C; Mycoplasma Assay Buffer at 4°C.

## Reagent and Sample Preparation

1. The mycoplasma detection reagent A and B are supplied as lyophilized pellets. They are reconstituted in the supplied Mycoplasma Assay Buffer to produce the working solution for use in the assay. For reconstitution, add 1 ml of Mycoplasma Assay Buffer to the Mycoplasma Detection Reagent A and B, respectively. Replace screw cap and mix gently. Allow equilibration to room temperature for 10 min. Unused components can be aliquoted and stored at -20°C.

2. Cell sample should be cultured for 3-6 days. Cell supernatant must be spun at 200×g for 5 min to remove any remaining cells or cell debris. For optimal assay performance, supernatant should be tested as soon as possible after collection. Supernatant can be kept at room temperature or 4°C for testing same day. Supernatant can be also frozen and stored at -80°C for 6 months. For assay frozen sample, thaw and equilibrate to room temperature for 15 min before testing.

## Protocol

1. Bring all reagents up to room temperature before use.
2. Reconstitute the Mycoplasma Detection Reagent A and B in Mycoplasma Assay Buffer. Leave for 10 minutes at room temperature to ensure complete rehydration.
3. Transfer 1 ml of cell culture or culture supernatant into a centrifuge tube and pellet any cells at 200×g for 5 minutes.
4. Transfer 50 µl of cleared supernatant into a luminometer cuvette/tube or well.
5. Program the luminometer to take a 1 second integrated reading.
6. Add 50 µl of Mycoplasma Detection Reagent A to each sample and wait 5 minutes.
7. Place cuvette or plate in luminometer and initiate the program (Reading A).
8. Add 50 µl of Mycoplasma Detection Reagent B to each sample and wait 10 minutes.
9. Place cuvette or plate in luminometer and initiate the program (Reading B).
10. Calculate ratio = Reading B/Reading A.

The ratio of Reading B to Reading A is used to determine whether a cell culture is contaminated by mycoplasma.

Ratio	Interpretation
< 0.9	Negative for mycoplasma
0.9-1.2	Borderline: quarantine cells & retest in 24 h
> 1.2	Mycoplasma contamination

The following mollicute species were tested using the kit.

Species	Origin/Source	Species	Origin/Source
<i>Acholeplasma laidlawii</i>	Mammalian/Avian	<i>Mycoplasma gallinaceum</i>	Mammalian/Avian
<i>Acholeplasma modicum</i>	Bovine	<i>Mycoplasma gallisepticum</i>	Avian
<i>Acholeplasma morum</i>	Mammalian	<i>Mycoplasma genitalium</i>	Human
<i>Mesoplasma entomophilum</i>	Insect	<i>Mycoplasma hominis</i>	Human
<i>Mesoplasma florum</i>	Plant/Insect	<i>Mycoplasma hyopneumoniae</i>	Human
<i>Mycoplasma agussizii</i>	Tortoise	<i>Mycoplasma hyorhinis</i>	Porcine
<i>Mycoplasma aikalescens</i>	Bovine	<i>Mycoplasma hyosynoviae</i>	Porcine
<i>Mycoplasma alligatoris</i>	Alligator	<i>Mycoplasma iguanae</i>	Iguana
<i>Mycoplasma arginini</i>	Bovine/Porcine	<i>Mycoplasma lipophilum</i>	Human
<i>Mycoplasma arthritis</i>	Human	<i>Mycoplasma muris</i>	Murine
<i>Mycoplasma bovirhinis</i>	Bovine	<i>Mycoplasma neurolyticum</i>	Murine
<i>Mycoplasma bovis</i>	Bovine	<i>Mycoplasma opalescens</i>	Canine
<i>Mycoplasma bovovuli</i>	Bovine	<i>Mycoplasma orale</i>	Human
<i>Mycoplasma buccale</i>	Human	<i>Mycoplasma pirum</i>	Human
<i>Mycoplasma californicum</i>	Bovine	<i>Mycoplasma pneumoniae</i>	Human
<i>Mycoplasma canadense</i>	Bovine	<i>Mycoplasma primatum</i>	Mammalian
<i>Mycoplasma cloacale</i>	Avian	<i>Mycoplasma pulmonis</i>	Human
<i>Mycoplasma conjunctivae</i>	Ovine & Caprine	<i>Mycoplasma pulmonis</i>	Rat
<i>Mycoplasma crocodyli</i>	Crocodile	<i>Mycoplasma salivarium</i>	Human
<i>Mycoplasma equirhinis</i>	Equine	<i>Mycoplasma spermatophilum</i>	Human
<i>Mycoplasma faucium</i>	Human	<i>Mycoplasma synoviae</i>	Avian
<i>Mycoplasma fermentans</i>	Human	<i>Spiroplasma citri</i>	Plant/Insect