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MycoCheck™ Mycoplasma Luminescent Detection Kit

Catalog Number: A066-1, A066-2

Table 1. Kit Components and Storage

Material	Amount	Storage	Stability
MycoCheck™ Mycoplasma Luminescent Detection Kit (Cat. No. A066-1)			The product is stable for one year when stored as directed.
Mycoplasma Detection Reagent A, lyophilized (Component A)	1 mL	-20 °C	
Mycoplasma Detection Reagent B, lyophilized (Component B)	1 mL		
Mycoplasma Assay Buffer (Component C)	10 mL	4 °C	
MycoCheck™ Mycoplasma Luminescent Detection Kit (Cat. No. A066-2)			
Mycoplasma Detection Reagent A, lyophilized (Component A)	5×1 mL	-20 °C	
Mycoplasma Detection Reagent B, lyophilized (Component B)	5×1 mL		
Mycoplasma Assay Buffer (Component C)	10 mL	4 °C	

Product Description

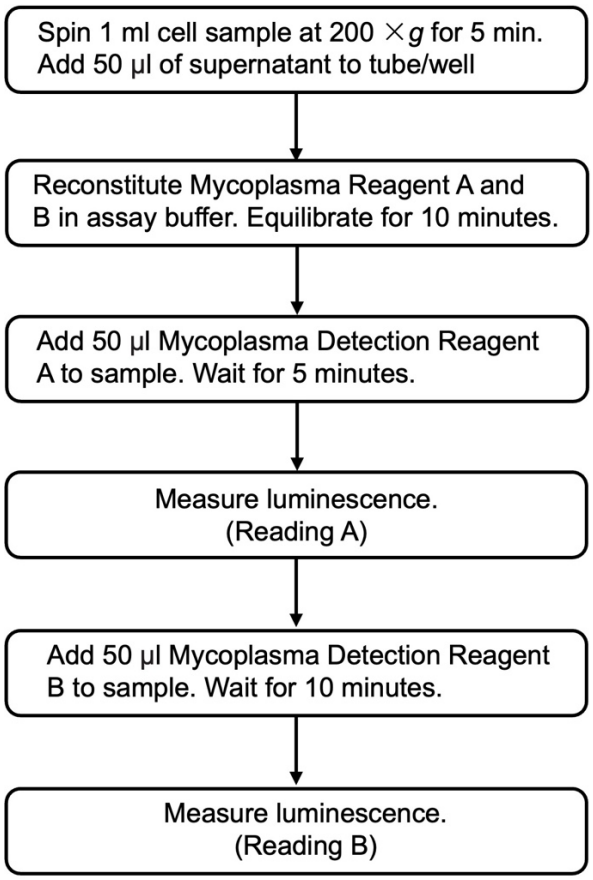
Mycoplasma infections are relatively common in laboratory cell cultures; it has been estimated that between 5% and 35% of all cell cultures are infected. Mycoplasma are the smallest and simplest prokaryotes. Mycoplasma depend on their hosts for many nutrients due to their limited biosynthetic capabilities. Mycoplasma have been shown to alter the growth rate of cells in culture, induce chromosomal aberrations, influence amino acid and nucleic acid metabolism and cause membrane aberrations.

The MycoCheck™ 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.

Equipment and consumables required but not provided

- Cuvette or plate luminometer
- Bench centrifuge
- Sterile centrifuge tubes
- Luminometer cuvettes or white walled microplates
- Micropipette and tips

Workflow diagram for the Mycoplasma Detection



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.

Assay 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 x *g* for 5 minutes.
4. Transfer 50 µl of cleared supernatant into a luminometer cuvette/tube or well.

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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.

Interpretation of results

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 contaminatio

MycoCheck™ tested species

The following mollicute species were tested using the MycoCheck™ Assay.

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