

## Mycoplasma Testing (April 2020)

### **MycoAlert Mycoplasma Detection kit Cat #: LT07-218 (25 tests)**

Kit includes:

1. LT27-217 MycoAlert Reagent. Lyophilized- 5 x 600 µl vials
2. LT27-218 MycoAlert Assay Buffer (store at 2-8 °C) - 1 x 10ml bottle.
3. LT27-221 MycoAlert Substrate. Lyophilized - 5 x 600 µl

### **MycoAlert Assay Control Set (10 tests) LT07-518**

#### **Reagents Reconstitution and Storage**

The MycoAlert Reagent and MycoAlert Substrate are supplied as lyophilized pellets. These are reconstituted in the MycoAlert Assay Buffer (supplied) to produce the working solutions for use in the assay.

**Use reconstituted Reagent and/or Substrate within 5 hours, or 5 days if stored at 2 °C – 8 °C. Unused components can be aliquotted into polypropylene tubes and stored at -20 °C for up to six months. Once thawed the reagent and/or substrate must not be refrozen and should be allowed to reach room temperature before use without the aid of artificial heat.**

#### 1. Preparation of MycoAlert Reagent

- Add 600 µl of the MycoAlert Assay Buffer into a vial containing the lyophilized MycoAlert Reagent.
- Replace the white screw cap and mix gently.
- Allow the reagent to equilibrate for 15 minutes at room temperature.

#### 2. Preparation of MycoAlert Substrate

- Add 600 µl of the MycoAlert Assay Buffer into a vial containing the lyophilized MycoAlert Substrate.
- Replace the green screw cap and mix gently.
- Allow the substrate to equilibrate for 15 minutes at room temperature.

#### 3. MycoAlert Assay Buffer

- This is provided ready for use. Store at 2 °C – 8 °C when not in use.

#### 4. Preparation of samples

- If HIV infected supernatant, boil samples at 100 °C for 5 min.
- For cell culture or culture supernatant, spin at 12,000 rpm for 5 min to get rid of cells pellet or debris.

**Protocol:**

1. Bring all reagents and samples to room temperature before use.
2. Reconstitute the MycoAlert Reagent and MycoAlert Substrate in MycoAlert Assay Buffer. Leave for 15 minutes at room temperature to ensure complete rehydration.
3. Transfer 100 µl of samples (clear supernatant) and controls (positive and negative) into a luminescence compatible plate. White walled plates provide best sensitivity with the MycoAlert Assay.
4. Program the luminometer to take a 1 second integrated reading.
5. Add 100 µl of MycoAlert Reagent to each sample and wait 5 minutes.
6. Seal top of plate and place in luminometer and initiate the program (Reading A).
7. Add 100 µl of MycoAlert Substrate to each sample and wait 10 minutes.
8. Place plate in luminometer and initiate the program (Reading B).
9. Calculate ratio = Reading B/Reading A.

**Interpretation of Results:**

The test has been designed to give ratios of less than 1 with uninfected cultures. Cells which are infected with mycoplasma will routinely produce ratios greater than 1.