

## SOP 6 Mycoplasma Assay

### Mycoplasma Assay

- 1) Thaw the Reagent and Substrate (100  $\mu$ L for each sample plus 1 positive and 1 negative control)
- 2) Thaw 1 positive control tube
- 3) Clean the Micropipettes with an alcohol pad and set the amount to 100 $\mu$ L
- 4) In your notebook, record each sample and assign each a corresponding number
- 5) Label each sample tube with its new corresponding number, along with a new clear 1.5mL microcentrifuge tube for each sample
- 6) Measure out 100  $\mu$ L of the each sample and place into the newly labeled coordinating tubes for testing (each of the controls should already be measured out to 100  $\mu$ L each and are ready for testing, if not see preparation of controls below)
- 7) Add 100  $\mu$ L of the "R" (Reagent) to each tube and each of the controls; MIX/SHAKE tube
- 8) Once the "R" has been added to each tube, place the rack OUT OF THE LIGHT and WAIT 5 minutes
  - o While Waiting, turn on the Luminometer (switch is located on the backside)
    - Select: "Measure"
- 9) After 5 minutes, calculate the Luminometer values by placing the positive control into the machine, close the door, wait the allotted time (2 second wait and 10 second exposure), record each value in your notebook and repeat with the negative control and each subsequent sample.
- 10) Next, add 100 $\mu$ L of the "S" (Substrate) to each tube and control; MIX/SHAKE tube
  - o Put the holding rack OUT OF THE LIGHT and WAIT 10 minutes
- 11) After 10 minutes, repeat the luminometer step 9 above
- 12) Once all values are obtained, discard all samples and any remaining reagents and/or substrate should be stored at 4°C for no longer than 1 week

### Calculating/Interpreting Results

- 1) Perform the following calculation to retrieve the values: value of 2<sup>nd</sup> Measurement /value of 1<sup>st</sup> Measurement = decimal number "n"
  - a. If  $n > 1$  = POSITIVE
  - b. If  $n < 1$  = NEGATIVE
  - c. Note: the positive control ratio should give an "n" of greater than 1, and the negative control ratio should give an "n" of less than 1
    - i. Results should be trusted only if the control results are within the acceptable range
- 2) Negative results are considered free of mycoplasma at an acceptable level
- 3) Positive results between a value of 1 and 2 should be retested with a new sample
  - a. False positives occur if the sample is stored longer than 1 week before testing or if light is on the samples, reagents, or substrates too long as this entire test is light-sensitive
- 4) Samples with positive results greater than 2 are at an unacceptable contamination level and coordinating samples should be removed from all incubators and thrown out.
  - a. For special circumstances, where samples cannot be tossed, a mycoplasma irradiation kit may be used. These however are not 100% successful and need to be conducted in a quarantined incubator. This kit is not an acceptable option for most projects and needs to be chosen with caution.

### Preparation of Reagent and Substrate (LT07-318)

- 1) Add 10 mL of the MycoAlert<sup>®</sup> Assay Buffer into the MycoAlert<sup>®</sup> Reagent (white top). Mix. Let stand for 15 min
- 2) Add 10 mL of the MycoAlert<sup>®</sup> Assay Buffer into the MycoAlert<sup>®</sup> Substrate (green top). Mix. Let stand for 15 min
- 3) While Reagent and Substrate are sitting, label 18 autoclaved clear 1.5 mL microcentrifuge tubes for the Reagent

with an "R" and the date, and 18 autoclaved clear 1.5mL microcentrifuge tubes for the Substrate with an "S" and the date.

- 4) After the 15 min wait, aliquot 550  $\mu$ L of both the Reagent and the Substrate into their corresponding new vials. Store at -20° C for up to 6 months.

#### Preparation of Positive Control (LT07-518)

- 1) Add 1mL of the MycoAlert® Assay Buffer into the MycoAlert® Assay Control (red top). Mix. Let stand for 15 min
- 2) Label 10 autoclaved clear 1.5 mL microcentrifuge tubes with "+ control" and the date
- 3) After the 15 min wait, aliquot 100  $\mu$ L into each new tube. Store at -20°C for up to 6 months.

#### Preparation of Negative Control

- 1) Label 10 autoclaved clear 1.5mL microcentrifuge tubes with "- control" and the date
- 2) Add 100  $\mu$ L of sterile water to each tube and store at 4°C