

# Checking for Mycoplasma via e-Myco *plus* PCR Kit

Updated 4/30/12 LA

Purchased from Boca Scientific

1. Collect 400ul culture in 1.5ml tube (store at 4°C, not -20°C)
2. Spin down 30 sec @ max speed, remove S/N
3. Resuspend pellet in 1ml sterile 1X PBS
4. Spin down 30 sec @ max speed, remove S/N
5. Resuspend in 100ul sterile 1X PBS
6. Heat at 95°C for 10 minutes (heat block)
7. Vortex 5 sec
8. Spin down 2 min, max speed
9. Add to PCR tubes from kit:

Sample	Negative (PBS)	Negative (H <sub>2</sub> O)	Positive
10ul H <sub>2</sub> O	20ul 1X PBS	20ul H <sub>2</sub> O	19ul H <sub>2</sub> O
10ul S/N from boiled sample	---	---	0.5ul myco DNA

10. Flick tubes to mix, spin down briefly
11. Run PCR (2 hours)
  - a. 94°C - 1 min
  - b. [94°C - 30 sec
  - c. 60°C – 20 sec
  - d. 72°C - 1 min] x 35 cycles
  - e. 72°C - 5 min
  - f. 4°C Hold
12. Run 5ul of each sample on 2% agarose gel with 1kb+ ladder (40 min, 100V)
  - a. 570 bp = sample control (may show up in positive control from kit, can ignore)
  - b. 260 bp = positive for mycoplasma
  - c. 160 bp = internal control (should be present for all samples and controls)
  - d. <100 bp = ignore