

**Condensed down Mycoplasma Test Protocol – reference protocol = MycoSensor PCR Assay Kit (Agilent Tech - #302109)**

**Extraction of Cell Culture Supernatant and Cells**

1. Set up heat block to 95°C
2. Take out 100 uL of media from test cell culture into a microcentrifuge tube
3. Scrape cells into 5 mL of 1X TC PBS and spin down 3 min at 1000 RPM in 15 mL centrifuge tube
4. Wash again with 1 mL 1X TC PBS and spin down 3 min at 1000 RPM
5. Resuspend again in 1 mL 1X TC PBS AND COUNT CELLS
6. Count out 100,000 cells into a new tube and again spin down (3 min at 1000 RPM)
7. Aspirate supe and add 100 uL of sterile dH<sub>2</sub>O
8. Boil tubes with 100 uL of media and cells in heating block set earlier. Media goes for 5 min. Cells go for 10 min.
9. Spin the boiled tubes briefly to get liquid off sides
10. Resuspend the kit-included StrataClean resin (**Green lid**) by vortexing the tube until all pellet is suspended in liquid
11. Add 10 uL of StrataClean to each tube and flick tubes to mix the resin and extracts
12. Spin all cells for 1 min to pellet the resin
13. Transfer supes to new tubes
14. Both can be stored at 4°C for several days

**PCR of the Samples**

SET UP:

RXN MM

| Component                                         | Volume Added (uL)   | 2 samples                  |
|---------------------------------------------------|---------------------|----------------------------|
| Sterile dH <sub>2</sub> O                         | 19.5                | 39 ✓                       |
| MgCl <sub>2</sub> (25 mM)                         | 8                   | 16 ✓                       |
| 5X Taq Buffer                                     | 10                  | 20 ✓                       |
| dNTP mix (from kit)                               | 2                   | 4 ✓                        |
| Mycoplasma Primer Mix (from kit – <b>red</b> lid) | 1                   | 2 ✓                        |
| Internal Ctrl template (from kit)                 | 4                   | 8 ✓                        |
| → Taq polymerase                                  | 0.5                 | 1                          |
| <b>RXN MM VOLUME</b>                              | <b>45 uL (each)</b> | <b>Template = add 5 uL</b> |

**TOTAL RXN VOLUME = 50 uL**

- Use Thermocycler program for Mycoplasma test (takes ~ 2 hours)
- Run on 2% agarose gel (0.8g agarose + 40 mL of NEW 1X TAE buffer) + 1.5 uL EtBr in small gel box
  - o Ladder Recipe: 1 uL LMW ladder + 2 uL 6X Loading Dye + 7 uL dH<sub>2</sub>O (TOT = 10 uL)
  - o Sample Recipe: 17 uL + 3 uL Loading Dye
  - o **Run at 120V for about 30 min.**