Protocol: Mycoplasma detection and treatment

Material

- Ciprofloxin (Sigma Aldrich, Cat.# 17850-5G-F)
- Ex Taq packet (Takara, Cat.# RR001A)
- Mycoplasma positive control template (PCR Mycoplasma detection set, Takara, Cat.# 6601)

Culturing Cells for Mycoplasma Detection

1. Thaw cells according to your specific lab protocols (usually in a T25). For Broad use only: if using our BL2+ space, please use the quarantine incubator located in room 5035.
2. When confluent (or about 90%), seed new T25 to at least 50% density.
3. Allow cells to grow to confluency.
4. After reaching 100% confluency, let cells sit in unchanged media for at least 3-5 days.
5. Remove 100ul – 1mL of media from flask and place in clean micro centrifuge tube or cryo vial.
6. For Broad use only, place sample in box labeled “Mycoplasma Test Box” on top shelf of -20°C freezer in room 5035.

Treating cell cultures contaminated with Mycoplasma

Ciprofloxin is used at a 10 ug/mL final concentration. It’s been suggested that this process will clear ~70% of cell lines from mycoplasma, kill ~10% of cell lines, and have no effect on the remaining ~20% of cell lines. Plan accordingly.

1. Cells are maintained in standard growth media supplemented with 10 ug/mL ciprofloxin for two weeks, with the standard passaging for the given cell line. If passages are >4 days apart, media should be replaced after three days with fresh growth media + ciprofloxin.
2. It is not uncommon for the cells to proliferate a little slower than usual and show morphological changes.
3. After two weeks of treatment, cells should be passaged for another two weeks in growth media alone without ciprofloxin (same passaging regimen).

4. After the four-week treatment, cells should be re-tested for contamination as above.

**Mycoplasma PCR Test**

1. Make master mix

   - H2O 30.5uL
   - 10x Ex Taq buffer 5uL
   - dNTP mixture 4uL
   - 10x primer mix (10 uM stock) 5uL
   - Ex Taq enzyme 0.5uL
   - **Total Volume (per sample)** 45uL

2. Spin media at high speed for 10 min to pellet any cells.
3. In a PCR plate, add 45 ul of master mix to each well.
4. Add 5 ul of media to each well.
5. Make a positive control by mixing 5 ul of positive control template with 45ul of master mix.
6. Mix well.
7. For Broad use: run “MYCOPLAS” program.

**MYCOPLASM thermal cycler protocol**

1. 94°C 2:00 minutes
2. 94°C 0:30
3. 55°C 2:00
4. 72°C 1:00

Repeat steps 2-4 for 34 cycles, then hold at 4°C.

**Primer sequences**

Forward - 5’ ACA CCA TGG GAG CTG GTA AT 3’

Reverse - 5’ CTT CWT CGA CTT YCA GAC CCA AGG CA 3’