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Protocol status: Working
We use this protocol and it's working

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Mycoplasma Removal Treatment Protocols

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DISCLAIMER

This protocols were not originally developed by the Lopez Lab.

ABSTRACT

This protocol describes three mycoplasma removal procedures to be utilized depending on need. The protocols are described from milder/maintenance to the harshest option to be used in extreme cases of contamination when it is not possible ato re-start the culture from a clear source.

MATERIALS

Mycoplasma Removal Agent, MP Biomedicals, cat #093050044 - 5 ml
(Stored at room temperature)

Plasmocin treatment agent, Invivogen, cat #ant-mpt Aliquot and Store at -20°C in TC freezer

Tiamulin, Santa Cruz, cat #sc-237107, (resuspend and dilute in 100% ethanol) Aliquot and Store at 4°C in TC fridge.

Minocycline Hydrochloride, Acros Organics, cat #455330010 Aliquot and Store at -20°C in TC
freezer

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Mycoplasma Treatment Protocols

1 NOTES for effective treatment

A. All treatment antibiotics should be added directly to the media at time of use. They should not be diluted or stored in media.

B. For the most effective treatment, the cells should be trypsinized when the media is changed to release any mycoplasma that is hiding/trapped between the cell and the plastic of the well.

C. If cells require splitting prior to the end of a given treatment, trypsinize and split as normal, replace the antibiotic and continue the same round of antibiotic treatment (ie, it does not count as a new round of treatment).

2 Cells from Lopez Lab cell stocks or trusted labs (previously confirmed Mycoplasma free):

1. Split cells very thinly (<20% confluent) in regular tissue culture media.
2. Add 10ul MRA per mL (up to 1mL total in a 6 wells plate).
3. Refresh media and MRA every 2 days over the course of 2-4 days (1-2 rounds of treatment).
4. Passage cells normally, freeze aliquots as needed.
5. Discard cells at 15 passages post treatment to prevent re-growth of mycoplasma.

3 Heavily contaminated cell lines or from labs with unknown mycoplasma testing procedures:

MRA treatment (Fluoroquinolone class antibiotic)

Reagent: Mycoplasma Removal Agent, MP Biomedicals, cat #093050044 - 5 ml
(Stored at room temperature)

1. Split cells very thinly (<20% confluent) in regular tissue culture media
2. Add 10ul MRA per mL (up to 1mL total in a 6 wells plate)
3. Refresh media and MRA every 2 days over the course of 8 days (4 rounds of treatment)
4. Test for mycoplasma (See **Mycoplasma Testing Protocol**)

***If cells are negative, they can be used normally (label cryo stocks as G1)
***If cells are borderline, move to **Step 4 – Plasmocin treatment**
***If cells are positive, go directly to **Step 5 – BM-Cyclin treatment**

4 **Plasmocin (Fluoroquinolone class antibiotic + Macrolide)**

Reagent: Plasmocin treatment agent, Invivogen, cat #ant-mpt Aliquot and Store at -20°C in TC freezer

1. Split cells very thinly (<20% confluent) in regular tissue culture media
2. Add 1ul Plasmocin (25mg/ml stock) per mL (up to 1mL total in a 6well plate) for a final concentration of 25ug/ml
3. Refresh media and Plasmocin every 2 days over the course of 14 days (7 rounds of treatment)
4. Re-test for mycoplasma (See **Mycoplasma Testing Protocol**)

***If cells are negative, they can be used normally (label cryo stocks as G1)
***If cells are still borderline or positive, go to **Step 5 – BM-Cyclin treatment**

5 **BM-Cyclin with Tiamulin and Minocycline (alternating Pleuromutilin+ Tetracycline class antibiotics)**

Reagents:

- *Tiamulin, Santa Cruz, cat #sc-237107, (resuspend and dilute in 100% ethanol) Aliquot and Store at 4°C in TC fridge.*
- *Minocycline Hydrochloride, Acros Organics, cat #455330010 Aliquot and Store at -20°C in TC freezer*

1. Split cells very thinly (<20% confluent) in regular tissue culture media
2. Add 4ul Tiamulin (2.5mg/ml stock) per mL (up to 1mL total in a 6well plate) for a final concentration of 10ug/ml
3. Incubate for 3 days
4. Trypsinize cells, wash with PBS, split as needed, and refresh media
5. Add 4ul Minocycline (1.25mg/ml stock) per mL (up to 1mL total in a 6well plate) for a final concentration of 5ug/ml
6. Incubate for 2 days
7. Refresh media and Minocycline, incubate for 2 days
8. Trypsinize cells, wash with PBS, split as needed, and refresh media
9. Repeat steps 2-7 twice more for a total of 21 days
10. Re-test for mycoplasma (See **Mycoplasma Testing Protocol**)

***If cells are negative, they can be used normally (label cryo stocks as G1)
***If cells are still borderline or positive, restart BM-Cyclin treatment