PurMa Biologics, LLC

PurMa™ Mycoplasma Treatment Reagent (MTR)(100X)
MTR I, MTR II & MTR III
User Manual

Initial Note:

For complete mycoplasma eradication, PurMa™ Mycoplasma Treatment Reagent (PurMa™ MTR-I) needs to be followed by PurMa™ MTR-II and later by PurMa™ MTR-III. This will eliminate most of the known mycoplasma species.

Protocol

1. In each well of a 96 well plate, pippete 20 µl of prepared spleen cells plus 180 µl medium containing 20% FBS, add 22 µl of PurMa ™ Antibiotic / Antimycotic Mix Solution (100X), High Fungicide (25 µg/ml) (Cat #: P3A111405)(Incubation Media).
2. Spin down the contaminated cells at 2000g for 5 min at room temperature. Avoid higher speed as that may collect some of the soluble mycoplasma strains.
3. Add 1 mL **PurMa™ MTR-I** to 9 ml of medium containing 20% FBS.
4. Count the contaminated cells and perform serial dilution in a way that there is NOT more than one in each well of the prepared 96 well plate. When this is performed, several wells will not receive any cells and some others get 2 or more cells. For the next, only the wells with one colony should be selected.
5. Incubate for 3 days.
6. Prepare a 96 well pate containing Incubation Media (see above), add 20 µl of diluted **PurMa™ MTR-II** to each well.
7. Based on the morphology of the colonies, pick the colonies from 5 wells from previous step. Use the cell count, and place one well of the 96 well plate containing PurMa™ MTR-II.
8. Incubate for 3 days.
9. Based on the morphology of the colonies, pick 10 wells, take 100 µl of each and place in 10 Eppendorf tubes. Perform PCR using PurMa™ Mycoplasma Detection PCR kit (Cat#: P5M011404)
10. At this stage, several wells show negative for mycoplasma.
11. It is imperative to separate the plate of the healthy colonies as soon as they are formed as failure to do so will cause cross contamination from the contaminated wells.

12. Prepare fresh a fresh 96 well plate containing Incubation Media (see above)
13. Dilute **PurMa™ MTR-II**I ten times and add 20 µl to each well of prepared 96 well plate.
14. Performing a cell count and serial dilution of the wells which showed no mycoplasma signal, take the volume which contains 1 cell and place in each well.
15. Incubate for 7 days.
16. At this stage, most of the wells should not have any mycoplasma contamination and the grow rate clearly will show that.
17. pick 10 wells, take 100 µl of each and place in 10 Eppendorf tubes. Perform PCR using PurMa™ Mycoplasma Detection PCR kit (Cat#: P5M011404)
18. transfer the content of wells with no mycoplasma signal in a fresh 12 well plate and add a few drops of fresh spleen cells.
19. Place the plate in your recently decontaminated CO2 incubator as at this stage, the cells are free of mycoplasma.
20. At this stage cells need an extra amount of feeder cells. Also, the first week after applying MTRI, II and III, an excessive amount of FBS (30%) is needed to help the cells overcome the healing process.
21. Incubate the cells for 5-7 days without changing the media.
22. For complete eradication, the above-mentioned procedure needs to be done twice as the mycoplasma could stay inactive inside the cells and get incorporated in the reproductive system after a period of dormancy.