

# Mycoplasma Testing for Cell Culture

## Sample Preparation from Cell Culture

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- Cultivate cells for a minimum of three days (72 h) without modifying the medium before collecting the supernatant for mycoplasma testing. Allow 80 - 90% confluence, but no higher, to avoid potential inhibition.

### Sampling:

- From adherent cell culture: collect supernatant directly from the culture flask.
- From suspension culture: collect cells at the bottom of the tube before removing the supernatant. Ensure supernatant is free of cells by centrifugation of a subsample.
- Collect 1000 µl of the cell-free supernatant in a screw cap tube or similar container that provides a tight seal during heat inactivation.
- Centrifuge the sample at 750 x g for 2 minutes to pellet remaining cells and cellular debris.
- Transfer **150 µl** of the supernatant to a new **1.5 ml flip-cap tube**.
- **Heat Inactivation:** Incubate the tube containing the supernatant at 95°C for 10 minutes.
- Label the tube with a Microsynth Mycoplasma barcode label. The label can be obtained in the webshop.

### Important:

- Samples with high viscosity are not suitable for the analysis and require DNA extraction.
- Presence of antibiotics (except penicillin and streptomycin) and other highly abundant substances may interfere with detection.

## Reducing Inhibition

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- Centrifuge 1000 µl of the collected supernatant at 750 x g for 2 minutes to pellet cells and cellular debris.
- Transfer the cleared supernatant, without touching the pellet, to a new **1.5 ml flip-cap tube** that will seal tightly during heat incubation.
- Centrifuge at  $\geq 15,000$  x g for 15 minutes.
- Carefully remove the supernatant and resuspend the (invisible) pellet in 100 µl of 10 mM TRIS-HCl, pH 7.5 buffer (or equivalent).
- **Heat Inactivation:** Incubate the test tube at 95°C for 10 minutes.
- Label the tube with a Microsynth Mycoplasma barcode label. The label can be obtained in the webshop.

## Other Sample Types (Cell Pellets, Cryostocks) and Persistently Inhibited Samples:

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- Perform DNA isolation using a commercially available DNA extraction kit.
- Transfer the isolated DNA into a **1.5 ml flip-cap tube**.
- Label the tube with a Microsynth Mycoplasma barcode label. The label can be obtained in the webshop.

### Important:

- Samples prepared differently or from a different source may contain matrix components that can interfere with the assay.
- If your samples are inhibited in our assay, please re-sample and use an appropriate DNA isolation kit or follow our suggestions to reduce the likelihood of inhibition.

## **Order Form Completion**

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Prior to shipping your samples to Microsynth, please proceed as follows to complete your order form:

- Enter our webshop on [www.microsynth.com](http://www.microsynth.com) (click on "LOGIN SHOP").
- Click on „Genetic Analysis“ in the green Analysis Services area.
- Click on "Tubes" under **Mycoplasma Testing for Cell Culture**.
- Fill in the order form and submit your order.
- Pack your samples into a transparent plastic bag (important: one bag per order) with the printed order form.
- Drop your sample package into the nearest Microsynth sample drop box (if available in your vicinity) or alternatively ship it by mail.

## **Need More Information?**

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### **Microsynth AG**

Schützenstrasse 15

9436 Balgach

Switzerland

Phone: +41 71 726 15 53

Email: [genotyping@microsynth.ch](mailto:genotyping@microsynth.ch)