Microsynth

Mycoplasma Testing for Cell Culture

Sample Preparation from Cell Culture

- 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 short, legible, and unique sample name on the tube wall.

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

- 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 ≥ 15,000 x g for 15 minutes.
- Carefully remove the supernatant and resuspend the (invisible) pellet in 100 µl of 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 short, legible, and unique sample name on the tube wall.

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

- 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 short, legible, and unique sample name on the tube wall.

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

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

- Enter our webshop on 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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