MYCOPLASMACHECK
Sample Preparation

For maximum sensitivity: Recommendations for cell culture cultivation prior to testing

- ☐ Cell lines should be cultured in the absence of mycoplasma-active antibiotics for one week prior to submission. Penicillin and streptomycin do not affect mycoplasma nor do they inhibit **MYCOPLASMA**CHECK.
- ☐ Cell cultures should be maintained in the same media without dilution with fresh media for three days before testing.
- ☐ Cryopreserved cell cultures should be cultured about two weeks prior to sample submission.
- ☐ 80-90% confluence of the tissue culture is recommended for testing. Inhibiting substances may accumulate in >90% confluent cultures.

Sample preparation of cell cultures:

1. **(a)** Transfer 500 µl of cell culture supernatant from the test cell culture to a 1.5 ml tube. The lid should be sealed tightly to prevent opening during heating.
   (b) For suspension cell lines, stand culture flasks vertically allowing cells to settle for about 30 minutes prior to removal of 500µL of the supernatant and transfer to a 1.5 ml tube.
2. Boil supernatant at 95 °C for 10 minutes.
3. Briefly centrifuge (5 seconds) the sample at ~13,000 rpm to pellet cellular debris.
4. Transfer 100 – 200 µl of supernatant into a new 1.5 ml tube labelled with a **MYCOPLASMA**CHECK barcode. Do not disturb pellet

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Sample preparation of cell cultures that are prone to PCR inhibition:

For adherent cell lines:

A) Transfer 500 µl of cell culture supernatant from the cell culture to a 1.5 ml tube.

B) Centrifuge sample for 10 min at >10.000 x g to pellet mycoplasma cells.

C) Discard 450 µL of supernatant without disturbing any pellet.

D) Boil remaining 50µL at 95 °C for 10 minutes. The lid should be sealed tightly to prevent opening during heating.

E) Perform DNA extraction using any commercially available DNA extraction kits according to the manufacturer´s instructions.

F) Elute in 30 µL using a 1.5 ml tube labelled with a MYCOPLASMACHECK barcode.

For suspension cell lines:

G) Centrifuge 500 µL sample for 3 min at >300 x g to pellet eucaryotic cells.

H) Transfer supernatant in new 1.5 ml tube.

I) Continue with step (B).

Testing of other non-cell-culture related material like cell pellets, fetal calf serum or cryo stocks:

- ☐ Those materials can be tested after DNA preparation by the customer using commercially available DNA extraction kits.
  e.g. QIAamp DNA Mini Kit (QIAGEN) or NucleoSpin® DNA RapidLyse (Macherey-Nagel)