



NCL Method STE-3

Detection of Mycoplasma

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This protocol assumes an intermediate level of scientific competency with regard to techniques, instrumentation, and safety procedures. Rudimentary assay details have been omitted for the sake of brevity.

Nanoparticles submitted to the NCL may be subjected to testing for mycoplasma when deemed necessary. Mycoplasma is a form of bacteria that lacks a nucleus and a cell wall, and are thus unaffected by many antibiotics. The types of nanoparticle formulations generally tested for mycoplasma contamination include those that incorporate a component derived from a bacterial culture, animal or hybridoma.

The NCL does not perform mycoplasma testing in our laboratory. Rather, this test is outsourced to another department located on the NCI-Frederick campus, the Protein Expression Laboratory (PEL), part of the Advanced Technology Program (<http://atp.ncifcrf.gov/pel/>).

Briefly, the NCL will culture nanoparticles with cells for the initial 24 hours, then passage these cells twenty five (25) times. These cells will then be transported to the PEL for mycoplasma testing via a pcr-based method. For more details on the mycoplasma detection protocol, please contact the PEL (<http://atp.ncifcrf.gov/pel/>).

Preparation of cells for mycoplasma testing:

1. Identify a quickly proliferating cell line (e.g. NCI H460).
2. Grow the cells in a T25 cell culture flask until they are approximately 80% confluent.
3. Add test nanoparticle formulation to the cell culture media, to a final concentration of 1 mg/mL (Note: the concentration may vary depending on the specific nanoformulation.).
4. Incubate cells for 24 hours, then replace growth medium with fresh complete medium appropriate for your cell line.
5. Split cells as needed and passage 25 times.
6. Use cells and supernatant for mycoplasma detection.

Note: this method is not intended to certify the nanoformulation as mycoplasma free. It is used to ensure that no mycoplasma is introduced into cell culture and transmitted to *in vitro* or *in vivo* (in case of xenograft studies) assays.