1193: CULTIVATION MEDIUM FOR CHLAMYDIAE

This recipe contains strain-specific modifications for *Chlamydia abortus* DSM 27654 *

Final volume: 50 ml

1. Cultivation of L929 (ACC 2) or HeLa (ACC 57) cells

IMDM-Medium	45.00	ml
Fetal bovine serum	5.00	ml
Aminoacids (100 x)	0.50	ml

2. Filter-sterilize (0.2 µm) and keep no longer than 4 weeks. Store at room temperature to facilitate detection of contamination.

3. Prepare a 25 cm2 flask and seed cells according to standard protocols (see DSMZ catalogue for ACC 2 and ACC 57). Incubate at 37°C plus 5% CO_2. When a confluent layer has formed, infection can be carried out.

4. Exchange medium with 6 ml of Infection Medium (as above with the addition of 1 µg/ml cycloheximide (final concentration)) and add 500 - 1000 µl of EB stock solution (thawed quickly to 37°C).

5. Centrifuge for 1 h onto the cell layer at 1600 rpm at 20°C.

6. Incubate at 37°C + 5% CO_2. Control cells daily and look for inclusions. Not all Chlamydiae form well-visible inclusions, ultimately, immunofluorescence or in situ-hybridization techniques are necessary to visualize inclusions.

* Plus 5% CO_2