

## 1193. CULTIVATION MEDIUM FOR CHLAMYDIAE

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

IMDM-Medium	45.0	ml
Fetal Bovine Serum	5.0	ml
Aminoacids (100 x)	0.5	ml

Filter-sterilize (0.2  $\mu\text{m}$ ) and keep no longer than 4 weeks. Store at room temperature to facilitate detection of contamination.

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

Exchange medium with 6 ml of Infection Medium (as above with the addition of 1  $\mu\text{g}/\text{ml}$  cycloheximide (final concentration)) and add 500 – 1000  $\mu\text{l}$  of EB stock solution (thawed quickly to 37°C).

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

Incubate at 37°C + 5% CO<sub>2</sub>. 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.