

## 1793: Chlamydia serpentis & poikilotherma Medium

MEM-Medium (+ Glutamine)	500.00	ml
Fetal bovine serum	100.00	ml
<b>D-glucose solution</b>	6.00	ml

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

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

<b>Cycloheximid solution</b>	0.70	ml
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3. Exchange medium with Infection Medium (as above with the addition of cycloheximide) and add 500 - 1000 µl of EB stock solution (thawed quickly to 37°C).

4. Centrifuge for 1 h onto the cell layer at 1000 x g at >20°C.

5. 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.

### D-glucose solution

D-Glucose	3.00	g
Demineralized water	50.00	ml

Filtrate.

### Cycloheximid solution

Cycloheximid	10.00	mg
Demineralized water	10.00	ml

Filtrate.