Main sol. 1512

1. Medium for maintenance of infected amoeba cultures

Solution A	800.00	ml
Solution B	50.00	ml
Solution C	50.00	ml

2. Combine Solutions A, B and C and add

Fetal bovine serum (heat-inactivated, 56°C, 30 min)	100.00	ml

3. Maintenance conditions:

4. Infected amoeba cultures can be maintained e.g. in 25 cm2 flasks (e.g. Nunc flasks, VWR, 734-2081, Thermo-Scientific, 156340) at 20-30°C in an incubator (no special requirements concerning CO_2 or light).

5. Maintenance/Passage of infected amoeba cultures:

6. Infected Acanthamoeba cultures don’t have to be transferred to fresh culture flasks as frequently as usually done for mammalian cell cultures. Amoeba cultures can be maintained in the same flask for several weeks or months. However, if the cultures reach a high cell density, medium should be exchanged. For that purpose amoebae are detached from the surface of the flask by knocking at the culture flask (no trypsin required, amoebae are only weakly adherent compared to most mammalian cells) and the medium (containing the majority of cells) is then completely removed. Fresh medium is added and the few remaining attached amoebae will continue to grow. Alternatively, cultures can also be passaged regularly (e.g. weekly) by transfer of a small aliquot of a densely grown culture (after cell detachment, as described above) to a fresh flask containing medium. If cultures contain few attached cells, but high amounts of cell debris, medium should be exchanged without prior cell detachment. Cultures usually recover fast. Depending on the host cell strain, the bacteria may cause host cell lysis. To maintain such cultures, bacteria have to be transferred by adding culture supernatant (approximately 0.5 ml) to fresh culture flasks containing uninfected host cells (approximately semi-confluent culture) each 7-10 days.