Main sol. 1514

1. Medium for maintenance of SF-9 cells (ACC 125) for cultivation of Rhabdochlamydiae.

Grace's Insect Medium (Sigma Aldrich, G8142)	900.00	ml
Fetal bovine serum (heat-inactivated, 56°C, 30 min)	100.00	ml

2. Filter-sterilize

3. Maintenance conditions of SF-9 cells (see also DSMZ catalogue for ACC 125):

4. SF-9 cells are cultivated at 25 - 29°C without the addition of CO_2 in flasks (25 cm2) with tightly closed lids. When a confluent layer has formed, infection can be carried out.

5. Infection with Rhabdochlamydiae:

6. Exchange medium and add 500 – 1000 µl of EB stock solution (thawed quickly to 37°C). Centrifuge for 1 h onto the cell layer at 1600 rpm at 20°C.

7. Maintenance/Passage of infected insect cell cultures:

8. Infected cultures can be maintained e.g. in 25 cm2 flasks (e.g. Nunc flasks, VWR, 734-2081) at 27°C in an incubator (no special requirements concerning CO_2 or light). If the percentage of infected cells is still low (which might occur initially after inoculation of cultures with bacteria from a frozen stock) infected cultures may be maintained by weekly passage (1:5). Infection of yet uninfected cells by added bacteria or bacteria released from neighboring infected cells is facilitated by centrifugation (130 x g, 15 min) of the culture flask. In well-infected cultures host cell lysis will be visible (the bacteria cause host cell lysis within approximately 5-7 days after invasion of host cells). To maintain such cultures, bacteria have to be transferred by adding culture supernatant (approximately 0.5-1 ml) to fresh culture flasks containing uninfected insect cells (approximately semi-confluent culture) each 7-10 days.