1311. XTC-2 MEDIUM

Cultivation of *Diplorickettsia massiliensis*

Cultivation of XTC-2 (from *Xenopus laevis*) cells

Leibovitz 15-Medium	93.0	ml
Fetal Bovine Serum	5.0	ml
Tryptose-phosphate	2.0	ml
Glutamine	2.0	mM (final concentration)

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

Prepare a 25 cm^2 flask and seed cells according to standard protocols. Incubate at 28 - 32°C in hermetic flasks. Cells are easily detached mechanically, no enzyme is necessary. Medium should be changed once a week. When a confluent layer has formed, infection can be carried out.

Infection medium:

Leibovitz 15-Medium	96.0	ml
Fetal Bovine Serum	2.0	ml
Tryptose-phosphate	2.0	ml
Glutamine	2.0	mM (final concentration)

Infect cells with 1 ml of *Diplorickettsia* stock solution (thawed quickly to 37°C). Incubate for 1 h at room temperature. **Cultivation temperature: 28°C.**

Up to 100% of the cells should be infected after several days. The infection level is evaluated after 3 days by Cytospin and Gimenez staining. Cytopathic effects may be visible after 3-5 days. Specific PCRs can be performed to verify the presence of diplorickettsiae.