

1198. Vero B4 Medium

Cultivation of Vero B4 (ACC 33) cells

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

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

Prepare a 25 cm² flask and seed cells according to standard protocols (see DSMZ catalogue for ACC 33). Incubate at 37°C plus 5% CO₂. When a confluent layer has formed, infection can be carried out.

Infect cells with 1 ml of *Rickettsia* stock solution (thawed quickly to 37°C).

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

Incubate at 28°C. Up to 90% of Vero cells should be infected after 15 days. Specific PCRs can be performed to verify the presence of rickettsiae. Ultimately, in situ-hybridization can be used to visualize them.