

**APPENDIX I: CRYOPRESERVATION**

1. Prepare a 30% (v/v) sterile glycerol solution in Alsever's solution (see below).
2. Dispense 0.5 mL of anticoagulant solution into a 15 mL test tube. Add to the anticoagulant blood collected by a facility-approved method from hamsters that had reached or are near peak parasitemia. Invert the tube several times to mix the blood with the anticoagulant.
3. In a separate test tube, add the heparinized blood dropwise to the 30% glycerol solution. Note that blood should be mixed with glycerol solution in a 2:1 ratio to obtain a final concentration of cryoprotectant of 10% (v/v). Mix slowly by inversion and place the tube on ice. The freezing process should start 15 to 30 minutes following the addition of the heparinized blood to the cryoprotectant solution.
4. Dispense 0.5 mL aliquots of blood suspension into 1 to 2 mL sterile plastic screw-capped cryovials. Place the vials in a controlled rate freezing unit. From room temperature, cool the vials at -1°C per min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C per min through this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
5. To thaw a frozen ampule, place in a 35°C to 37°C water bath, until thawed (2 to 3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
6. Immediately after thawing, remove the contents of the ampule aseptically with a syringe and inoculate a naïve hamster. Follow the protocol for *in vivo* propagation in the Growth Conditions section of the Product Information Sheet.

Alsever's Solution<sup>1</sup>

Sodium chloride	4.2 g
Trisodium citrate dihydrate (Na <sub>3</sub> citrate • 2H <sub>2</sub> O)	8 g
Glucose	20.5 g
Glass-distilled H <sub>2</sub> O to	1 L

Dissolve components in glass distilled H<sub>2</sub>O, adjust the pH to 6.1 with 10% (w/v) citric acid and filter sterilize.

<sup>1</sup>This solution can be obtained from Sigma-Aldrich® (catalog number A3551).

Yaeger's Anticoagulant Solution

Sodium citrate	1.33 g
Citric acid	0.47 g
Dextrose	3 g
Sodium heparin	0.2 g
Distilled H <sub>2</sub> O to	100 mL