KALADRUG-R: Laboratory SOP #5

Cryopreservation *L. donovani* promastigote cultures

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A. Material required

1. Mr. Frosty
2. Sterile cryovials + liquid nitrogen resistant labels
3. Sterile universals
4. 10 ML syringe + 19 ½ gauge needle

B. Solutions required: sterile 30% glycerol in PBS

1. Prepare a 30% glycerol-PBS solution
2. Filter sterilise under pressure in Millipore Stericup
3. Store at 4°C

PBS (Phosphate-buffered saline pH=7.4)	
bidistilled water	1000 ml
NaCl	7.2 g
$Na_2HPO_4.2H2O$	1.854 g
KH_2PO_4	0.43 g
pH adjusted to 7.4 with HCl or NaOH 1N	
Sterilize through a 0.22 µm membrane filter	

C. Instructions for use of Mr. Frosty

1. Remove the vial holder and foam insert from the container. DO NOT discard foam insert.
2. Add 100% isopropyl alcohol (250ml) to fill until the line. DO NOT OVERFILL

3. Carefully replace foam insert and vial holder. The container has to stay on room temperature, when not in use.
4. Replace the isopropyl alcohol every fifth use.

D. Cryopreserving *L. donovani* promastigote cultures

1. Use parasite culture at mid log-early stationary phase and inspect for growth and good culture conditions
2. Make a parasite culture dilution in 30% glycerol/PBS to fill all cryovials of your stabilate batch: 2/3 parasite culture + 1/3 30% glycerol/PBS in a sterile universal (end concentration= 10% glycerol).
3. Mix with vortex (glycerol needs to mix properly with parasites)
4. You can use a syringe + 19 ½ gauge needle to aspirate the glycerol/parasite suspension from the sterile container and subsequently distribute 1 mL of the parasite dilution to each CLEARLY LABELLED cryovial in a sterile manner (see SOP #13 for labeling)
5. Close the cryovials firmly and put them in a Mr. Frosty ready for use (Mr. Frosty should be at room temperature, filled with recommended amount of isopropanol)
6. Transfer the Mr. Frosty containing all prepared cyrovials to -70°C
7. After min 48hrs, transfer the cryovials to liquid nitrogen container or -70°C: distribute the cryovials of 1 stabilate over at least 2 containers or -70°C freezers, to protect loss of an entire stabilate in case of container breakdown or power failure
8. Register the new stabilates in your stabilate register
9. Quality control of stabilates: take out 1 vial of your stabilate after it has been frozen in liquid nitrogen or stored at -70°C and start a culture as described in SOP#11. If the resulting culture picks up good growth, the stabilate batch has passed quality control.