



KM-13 Glycerol-Buffer

- **Blood samples**

should be taken into a sterile heparinized tube. Blood with at least 0,5 % parasitaemia is suitable for cryopreservation.

- **Centrifugation**

Samples are centrifuged at 800-1000xg for 5 min and then plasma and buffy coat are removed. The packed red cells should become washed with RPMI1640(or PBS or saline) by centrifugation at 800-1000xg for 5 min three times. When the supernatant is discarded the pellet should become transferred to a 50ml sterile conical tube.

- **Freezing samples**

- Measure the volume of packed red blood cells.

- Calculate the first volume of Glycerol-Buffer to be added into the packed red cells:

- The first volume of Glycerol-Buffer should be 1/3 of the volume of packed red blood cells.

- Add the first volume of Glycerol-Buffer into packed red blood cells very slowly, adding one drop at a time with very gentle and continuous mixing.

☞ This step should take at least 3 - 5 min.

- Stand for 5 min at room temperature to allow the Glycerol to permeate the cells.

- The second volume of Glycerol-Buffer to become added is 1,33 x volume.

- Add the second volume of Glycerol-Buffer into the packed red cells by adding the Glycerol-Buffer still one drop at a time with gentle and continuous mixing.

☞ This step should take at least 3 - 5 min.

- Transfer the cell suspension into a cryotube.

- Keep at -80°C overnight then transfer to liquid Nitrogen for long term storage

- **Precautions:** All centrifugation/washing steps should be performed at +4°C !