

Aliquoting and Preparation of 0.25mM & 1.0mM reduced glutathione (GSH)

1.0 Equipment:

- 1.1** Analytical Balance
- 1.2** Spatula
- 1.3** 10ml pipette
- 1.4** 200ul pipette
- 1.5** 20ul pipette
- 1.6** Falcon tube rack
- 1.7** Ice bucket

2.0 Supplies:

- 2.1** Weighing papers/boats
- 2.2** 1.5ml Eppendorfs
- 2.3** Tissues
- 2.4** Purified water
- 2.5** Permanent black marker pen
- 2.6** 14ml Falcon Tubes
- 2.7** Human Tubal Fluid (hTF)
- 2.8** 0.2µm filters
- 2.9** 10ml syringe
- 2.10** 1-200ul Pipette tips
- 2.11** 10ml Diamond Pipette tips
- 2.12** Parafilm

3.0 Procedure

3.1 Aliquoting GSH

3.1.1 Print out labels with the following information; 'GSH', Lot No. and date, then label the Eppendorfs.

3.1.2 Fill the ice bucket with a layer of ice on the base and up the sides so that you can place the bottle of GSH inside and be able to fit the lid on comfortably.

NOTE: GSH is kept in the fridge and by weighing out such small amounts it can warm up quickly; therefore it is necessary to keep it cool in the ice bucket.

3.1.3 Wipe down the analytical balance after use to maintain this piece of equipment.

3.1.4 Clean the spatula with purified water and dry with a tissue before and after use.

3.1.5 Remove GSH bottle from the fridge and place inside the ice bucket. Place the weighing vessel/paper on balance and press the "tare" button to zero it.

3.1.6 Using the spatula weigh out 0.0307g (0.0304-0.0309g is acceptable) of GSH powder onto the weighing vessel/paper.

NOTE: Do not put GSH back into the bottle after it has been removed. If there is an excess of GSH put it in another weighing vessel and it can be used for the next Eppendorf.

3.1.7 Carefully pick up the weighing vessel and tip the powder into a labelled Eppendorf making sure not to spill any.

3.1.8 Store at 4°C.

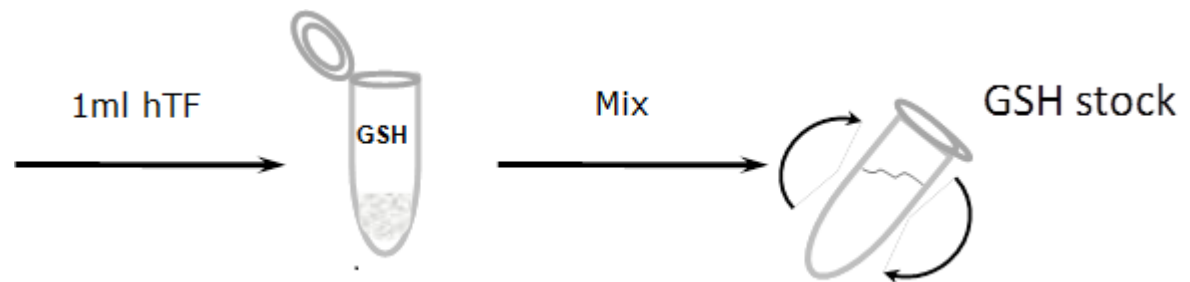
3.1.9 Repeat steps 3.1.5 and 3.1.8 until you have the number of aliquots required.

3.1.10 Clean analytical balance after use with 70% alcohol.

3.2 Preparing GSH stock solution

- 3.2.1 Take an Eppendorf containing the weighed out GSH from the fridge and add 1ml of hTF to the GSH. Close the lid and invert the tube several times to ensure the powder has fully dissolved (Picture 1). This is the GSH stock solution.

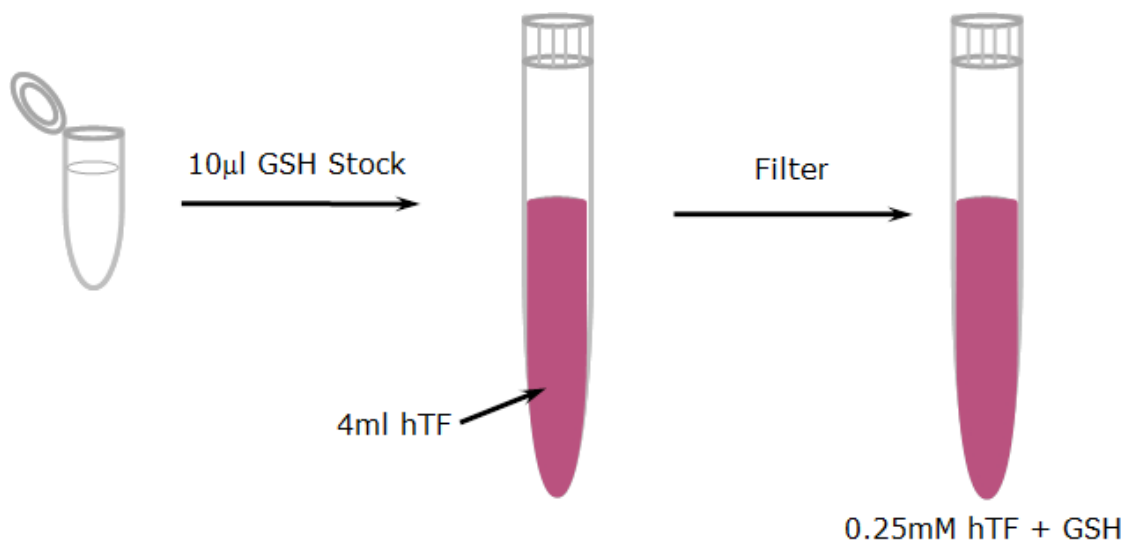
Picture 1



3.3 Preparing 0.25mM of GSH for FRESH sperm samples

- 3.3.1 Aliquot 4ml of hTF into a 14ml Falcon tube. Add 10 μ l of GSH stock solution to the 4ml of hTF. Replace the lid and invert several times to ensure the reagents have mixed sufficiently (Picture 2).

Picture 2



- 3.3.2 Remove the filter pack lid without touching the filter, leaving it inside the plastic casing.

- 3.3.3 Pull the plunger out of the disposable syringe, taking care not to let anything come into contact with the end of the plunger that comes into contact with the media.
- 3.3.4 Attach the syringe to the filter, and pour the hTF into the syringe.
- 3.3.5 Replace the plunger carefully, expelling the first few drops before filtering the rest into a new falcon tube. Make sure that the filter does not come into contact with anything when filtering the hTF.
- 3.3.6 Write on the new tube with permanent marker "FRESH" and the date.

3.4 Preparing 1.0mM of GSH for FROZEN sperm samples

- 3.4.1 Aliquot 5ml of hTF into a 14ml Eppendorf. Add 50 μ l of GSH stock solution to the 5ml of hTF. Replace the lid and invert several times to ensure the reagents have mixed sufficiently (Picture 3). Follow steps 3.3.2 – 3.3.5 to filter the hTF + GSH.
- 3.4.2 Write on the new tube with permanent marker "FROZEN" and the date.

Picture 3

