NXR Slow-Freezing Cryoprotectant Protocol

Cryoprotectant

0.4M sucrose…… 136.92g
10mM $NaHCO_3$…… 0.84g
2mM pentoxifylline…… 0.56g
1000mL → **40mL**

Vacuum filter

Organic Egg Yolk…… ~17.5mL
MQ H_20……~17.5mL
35mL → 1:1 → **10mL**

40mL + **10mL** = **50mL**

200mM L-glutamine stock:

29.23mg/mL L-glutamine

- Filter sterilize.
- Aliquot (15-20µL aliquots).
- Store at -20°C.

L-15+FBS+L-glutamine:

L-15……900µL
10% Fetal bovine serum (FBS)……..100µL - Heat inactivated FBS, store at -20°C
L-glutamine 200mM……10µL
1mL

Prepare for single use. Keep cold until use.

(Cryoprotectant)

1. Measure and combine the sucrose, sodium bicarbonate, and pentoxifylline in a beaker.
2. Fill the beaker to 1000mL with ultrapure H_2O.
3. Stir the solution until combined.
4. Filter the solution through a 0.2µm sterile vacuum filter.

   It should take ~1 minute to filter the entire volume.
5. Collect a fresh chicken egg, separate the yolk, and discard the egg whites.

   Best results have been observed when using fresh eggs. Where possible, obtain eggs from local sources rather than grocery stores where eggs may be 1-2 months old by the time of sale.
6. Add the yolk to a 50mL tube and note the volume occupied by the egg.

   Agitate the tube so that the egg rests in the bottom of the tube to ensure an accurate measurement.
7. Add a volume of ultrapure H_2O to the 50mL tube equal to the volume of the egg yolk.
8. Vortex the egg yolk mixture until combined but not frothing.
9. Add the sucrose solution (40mL) to the egg yolk mixture (10mL).
10. Divide into centrifuge tubes.

    The mixture can be centrifuged in 2mL, or up to 50mL centrifuge tubes.
11. Centrifuge the mixture at 10,000g (9100 rcf) for 20 minutes at 10°C.
12. Once the cycle has finished, remove the centrifuge tubes and pour the supernatant into a 50mL tube.

    Discard the centrifuge tubes including remaining pellets.
13. Aliquot the supernatant in 1.2-1.5mL volumes and place in a -80°C freezer for 3+ hours.
14. Once frozen, the aliquots can be stored short-term at -20°C or long-term at -80°C.

    Short-term storage can be considered ~2-3 months.
    Long-term storage can be considered ~12 months.
15. Aliquots should be discarded if a drop in quality is observed.