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Standard Operating Procedure (SOP) 013V3.0

Cryopreservation of Normal Breast Tissue

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Materials:

Lonza BioWhittaker Cryoprotective™ Media (Fisher 12-132-A)

Y-27632 Inhibitor (ROCK Inhibitor) (Enzo Life Sciences ALX-270-333-M001, for the 1mg or ALX-270-333-M005 for the 5mg)

DMEM F-12 (Life Technologies11765-054)

DMEM low glucose (Life Technologies 11-885-084)

Fetal Bovine Serum (FBS) Sigma F6178-50ml

Hydrocortisone (Sigma) H0888

Penicillin-Streptomycin (Sigma P4333-20ml)

Insulin (Sigma I6634-50mg)

1N HCl (Sigma H9892)

Bovine serum Albumin (BSA) Sigma A4503

HyClone™ Phosphate Buffered Saline (PBS) 1X (Fisher SH3025601)

Epidermal growth factor (EGF), human recombinant (Millipore 01-107)

Syringe (Fisher 14-823-24)

0.45µm Syringe Filter (Fisher 09-719B)

Sterile Forceps (VWR 12576-934)

Scalpels #11 (Fisher 08-927-5B)

Brown Eppendorf tubes (Fisher 05-408-134)

CoolCell FTS30 (Biocision BCS-170)

Isopropyl alcohol C3H8O 100% (Santa Cruz Biotechnology Inc. sc361622)

Petri Dishes (VWR 25383-166)

Preparation of Media

The amount of media needed for a typical collection event is as follows:

Cryoprotective (collection) media: **120 samples- 600ml** (5ml per conical tube).
Freezing media: **120 samples**- 3 aliquots for each sample= **360ml**

To prepare Freezing media (make fresh on the morning of the event):
Mix together: 180ml cryoprotective media + 90 µl Rock Inhibitor + 180ml growth media.
To each cryovial add 1mL freezing media.

Growth Media (total of 530.255mL):

1) DMEM F-12: 375ml – **use as is**
2) DMEM: 125 ml – **use as is**
3) FBS: 25 ml – **use as is**
4) Pen/Strep: 5ml – **use as is**
5) Insulin (1mg/ml): 2.5ml – **make stock (1)**
6) Hydrocortisone (1mg/ml): 250µl – **make stock (3)**
7) EGF from Millipore: 10µl of 1µg/µl – **make stock (2)** add a little media from steps 1-6 to EGF stock. Mix by pipetting and return solution to media.

Filter the mix through 0.22µm 500ml filter (Corning cat# 431097)
Store the sterile media at 4°C

Freezing Media- 360ml (make fresh on the morning of the event)

50% Growth Media + Rock Inhibitor and 50% Cryoprotective media.

Add 90µl of Rock Inhibitor (from stock of 10mM) to 180ml Cryoprotective media and mix. Add 180ml Freezing media and mix. Aliquot 1ml into each cryotube ready for the tissue to be added.

Rock Inhibitor – Make Stock (10mM)

Comes as 5mg, store at -20°C until use
Add 1.56ml of sterile water to the 5mg powder
Mix by pipetting
Aliquot into 300µl aliquots in brown Eppendorf tubes
Store aliquots at -20°C.

Stock Solutions for Growth Media

1) Insulin (1mg/ml), make 10ml

1% BSA/PBS stock solution

Dissolve 500mg of BSA in 50ml PBS.
Filter sterilize inside hood
Store at 4°C

0.1% BSA/PBS stock solution

45ml of sterile PBS + 5ml of 1% BSA/PBS. Use this to make insulin. Store at 4°C

Dissolve 10mg of insulin in 10 ml of 0.1%BSA/PBS.
Add 100µl of 1N HCl per 10ml of solution. Filter sterilize using 0.45µm filter.
Store at 4°C

2) EGF (1µg/µl)

100µl sterile water into 100µg of EGF (add water into the vial that the EGF comes in)
Make 10µl aliquots
Store at -80°C

3) Hydrocortisone (1mg/ml)

Dissolve 1mg of hydrocortisone in 1ml 100% EtOH
Make 250µl aliquots
Store at -20°C

Cryopreservation of Breast Tissue Cores

- A core is chosen from a group of cores brought to the lab area.
- Immediately place the chosen core in a 50ml conical tube that has Lonza BioWhittaker Cryoprotective™ Media in it. **Be sure the label on the conical tube matches the label that came with the core.**
- The conical tube and the 3 matching, pre-labelled cryotubes are given to the tissue preserver.
- Aliquot 1ml of **freezing** media into each cryotube.
- In a sterile environment, pour the collection media and core sample into a sterile petri dish. With a sterile scalpel, cut the core into small pieces (<2mm).
- Using a sterile forceps put 1/3 of the cut pieces into each of the three labelled cryotubes.
- Discard media remaining in petri dish into a media waste bottle.

- Place the cryotubes with tissue and freezing media into a slot in a **CoolCell FTS30** Cryo Container. Once the cryocontainer is full (30 tubes, 10 donor samples) it is placed into dry ice until it can be transferred to the -80°C freezer. The tubes are transferred to vapor phase after at least 4 hours being stored in -80°C or within one week.

Bibliography

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Resources

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