susan g. komen | TISSUE BANK at the IU SIMON CANCER CENTER

Standard Operating Procedure (SOP) 013V3.0

Cryopreservation of Normal Breast Tissue

Effective Date: September 10, 2015
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Approved by:

Materials:

Lonza BioWhittaker Cryoprotective™ Media (Fisher12-132-A)

Y-27632 Inhibitor (ROCK Inhibitor) (Fisher NC9858146)

DMEM F-12 (Life technologies11765-054)

DMEM low glucose (Life Technologies 12320-032)

Fetal Bovine Serum (FBS) Sigma F6178-50ml

Hydrocortisone (Sigma) H0888

Penicillin-Streptomycin (Sigma P4333-20ml)

Insulin (Sigma I5500)

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)

.45um Syringe Filter (Fisher 09-719B)

Sterile Forceps (Fisher NC9812170)

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

Brown Eppendorf tubes (Fisher 05-408-134)

Mr. Frosty freezing container (VWR 55710-200)

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

Petri Dishes (Fisher 08-772-E)

Preparation of Media

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

60 samples- 300ml cryoprotective (collection) media. (5mls per conical tube)
60 samples = 180 aliquots = 180mls Freezing media: Mix together: 150 mls cryoprotect media
150 mls growth media
300 ul Rock Inhibitor

Growth Media for primary cells- 530.255ml

1) DMEM F-12: 375ml – **use as is**
2) DMEM: 125 ml – **use as is**
3) FBS: 25 ml – **use as is-store at -20**
4) Pen/Strep: 5ml -- **Use as is**
5) Insulin (1mg/ml): 2.5ml – **Make Stock (1)**
6) Hydrocortisone (1mg/ml): 250ul – **Make Stock (3)**
7) EGF from Millipore: 5ul of 2ug/ul – **Make Stock (2)** add a little media from steps 1-6 to EGF stock. Mix by pipetting and return solution to media.

Freezing Media- 300ml

50/50 Growth Media and Cryoprotective media + Rock Inhibitor
Mix 150ml Cryoprotective media with 150ml Freezing media. Add 300ul of Rock Inhibitor. (10mMol)
Store overnight at 4C before use.
Aliquot 1ml into each cryotube ready for tissue.

Rock Inhibitor – Make Stock

Comes as 5mg, store at -20 until use
Add 1.56ml of sterile water (autoclaved DI water) to the 5mg
Mix by pipetting
Aliquot into 300ul aliquots in brown Eppendorf tubes
Store aliquots at -20.

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Stock Solutions for Growth Media

1)Insulin: Make 10 ml

1% BSA/PBS stock solution

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

.1% BSA/PBS stock solution

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

Insulin

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

2) EGF

100ug of EGF into 50ul sterile water (autoclaved DI water)
Make 5ul aliquots
Store at -80

3) Hydrocortisone

Dissolve 1mg of hydrocortisone in 1ml 100% EtOH
Make 250ul aliquots
Store at 4C

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.

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- Place the cryotubes with tissue and freezing media into a slot in a Nalgene Mr. Frosty Cryo Container. The container should previously have had 250ml of isopropyl alcohol added to it according to manufacturer's instructions. Once the cryocontainer is full (18 tubes, 6 donor samples) it is placed into dry ice until it can be transferred to the -80 freezer . The tubes are transferred to vapor phase of LN2 the next day or within one week.

Bibliography

- Liu X., Ory V., et al. ROCK Inhibitor and Feeder Cells Induce the Conditional Reprogramming of Epithelial Cells. American Journal of Pathology, Vol. 180 No.2, February 2012
- Hubel A.,Spindler R., Skubitz A. Storage of Human Biospecimens: Selection of the Optimal Storage Temperature. Biopreservation and Biobanking, Vol. 12 No. 3 2012.

Resources

Personal communication with Hari Nakshatri B.V.Sc., Ph.D. **Marian J. Morrison Professor in Breast Cancer Research,**IU School of Medicine; **Professor,** Department of Surgery
IU School of Medicine; **Professor,** Department of Biochemistry and Molecular Biology
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Personal communication with Poornima Nakshatri and Manju Anjanappa MS Research Technician.

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