SOP_MTL-1.2 Freezing Media

A. Purpose: To provide aseptic techniques and instructions to prepare freezing media.

B. Scope: The procedure described here can be used to make media to viably freeze tissue from solid tumors and cell suspensions.

C. Definitions:

DMEM: Dulbecco's modified Eagle medium

DMSO: Dimethyl sulfoxide

EtOH: Ethanol

FBS: Fetal bovine serum

Freezing media: 50% FBS, 40% DMEM, 10% DMSO

D. Materials and Reagents: In quantities to prepare 500 mL of freezing media.

Name	Quantity	Cat number	Sterility status for use
25 mL Serological pipette	1	P7865, Greiner	Sterile
50 mL Conical tubes	10-15	14-959-49A, Fisher scientific	Sterile
50 mL Serological pipette	4-5	Z652555, Greiner	Sterile
500 mL Bottle-top vacuum filter system (0.2 mm)	1	CLS430758, Corning	Sterile
70% Ethanol spray bottle	1	LC222102, Fisher scientific	Non-sterile
DMEM	200 mL	11330-032, Thermofisher	Sterile
DMSO	50 mL	D8418-50ML, Sigma	Sterile
FBS	250 mL	SH3007103, Hyclone	Sterile
Paper towels	Several	10714-002, VWR	Non-sterile
Pipet-aid	1	4-000-101, Drummond	Sterile
Vacuum system	1	NA	Sterile

E. References: NA

F. Procedures:

General Considerations:

Media preparation should be done inside the cell culture hood, following standard sterile procedures. Use aseptic techniques throughout the procedure. All items going into the cell culture hood should be sprayed with 70% ethanol and wiped down with a paper towel.

1. Thaw the FBS in the 37°C bead bath in the cell culture room.
2. Obtain a 500 mL 0.2 μm bottle-top vacuum filter system and place it in the hood.

3. Open the 500 mL filter bottle system:
   3.1. Remove the lid of the top part of the system, place it face down until it is needed.
   3.2. Attach the vacuum extension piece to the outside of the filter so the vacuum tubing will fit.
4. Check the FBS. Once it is fully thawed, mix thoroughly and place it in the hood.
5. Using a 50 mL serological pipette, transfer 200 mL of DMEM onto the filter. Discard the pipette.
   5.1. Place the lid back on the filter after the addition of each component to help avoid spilling and contamination.
   5.2. Going forward, be careful not to tip the filter bottle as it is now very top heavy.
6. Using a 50 mL serological pipette, transfer 250 mL of FBS to the DMEM. Discard the pipette.
7. Using a 25 mL serological pipette, transfer 50 mL of DMSO to the DMEM/FBS. Discard the pipette.
   7.1. Make sure to add the DMSO last because it will dissolve the filter if added first.
8. Attach the vacuum hose to the nozzle on the side of the filter and turn on the vacuum.
9. Once all of the media has filtered through to the bottom bottle, unscrew the filter and dispose of it in the biohazard trash.
10. Using a 50 mL pipette, aliquot 50 mL of the freezing media into ten 50 mL Conical tubes.
11. Label the tubes with the media formulation, date the media was made, and the expiration date. The expiration date is the most recent expiration date of either the FBS, DMEM, or DMSO.
12. Store freezing media aliquots in -20°C freezer.
13. If there is any remaining FBS, use a 50 mL pipette to make 25-50 mL aliquots into 50 mL Conical tubes. Label the tubes and include the expiration date.
14. Store FBS aliquots in -20°C freezer.

G. Revisions log:

Version	Revision Date	Section Revised	Notes
1	10.16.2020	All	SOP created
2	01.22.2021	D	Format updated