Recommended Cryopreservation Protocol for hMSCs

Protocol Summary

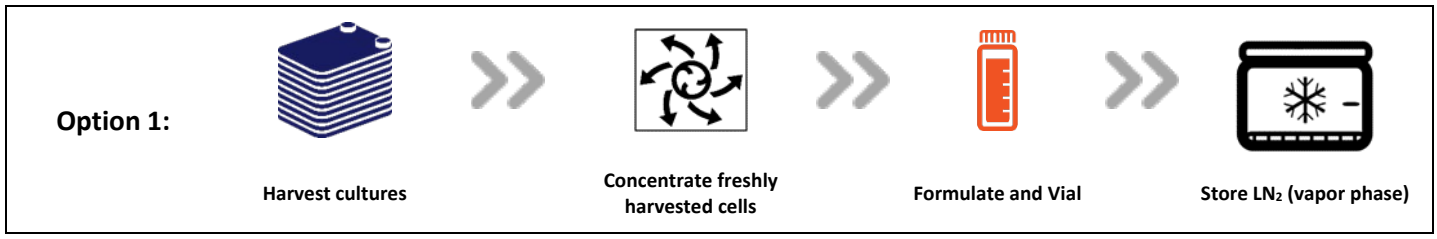
The general process recommendations to maintain cell viability when cryopreserving hMSCs are outlined below.

Materials & Equipment

Item	Vendor	Part Number*
RoosterNourish™	RoosterBio	K82003/K82016
TrypLE Select Enzyme	Life Technologies	12563029
CryoStor CS5	BioLife Solutions	205373/205102/205202
Biosafety Cabinet		
Centrifuge		
Incubator		
Freezing container/ Controlled Rate Freezer		
Liquid Nitrogen Dewar		

Process Overview

Cryopreservation of hMSCs for Storage



**RoosterBio strongly recommends the use of Corning CellBIND surfaces for expansion of the Xeno-Free product line.*
***Please refer to protocol for full process instructions.*

- Harvest cells using TrypLE, dilute in RoosterNourish, and transfer to centrifuge conical.
- Centrifuge/concentrate freshly harvested cells.
- Aspirate supernatant without disrupting cell pellet.
- Resuspend directly in CryoStor5 at concentrations 1-20M viable cells/mL.
- Cryopreserve with controlled rate freezing (~1°C/min) until -80°C then transfer to liquid nitrogen (vapor phase).

Recommended Protocol

1. Media Preparation

1.1. Bring RoosterNourish-MSC-XF components to room temperature, protected from light, for up to four hours.
1.2. Prepare bottles of medium by aseptically adding bottles of RoosterBooster™-MSC(-XF) (Part No. SU-003/016) to bottles of RoosterBasal™2.0-CC (Part No. M22520).
1.3. Mix well by capping and gently mixing the bottle.

2. Cell Harvest

2.1. For harvest, transfer vessels into biosafety cabinet and remove spent media.
2.2. Add appropriate amount of (mL) TrypLE to each vessel as indicated in the table below:

Working Volume Table (mL)					
	T25	**T75**	**T225**	**CellStack 5**	**CellStack 10**
Media Volume	5	15	45	750	1500
TrypLE Volume	1	3	10	100	200
Surface Area (cm^2)	25	75	225	3180	6360

2.3. Distribute TrypLE evenly to cover all the cells and place vessels in 37°C (5% CO2) incubator. Check culture every 5 min until cells are detached from surface. Gently tap to dislodge remaining cells from surface.

Total Time Required for Cell Detachment

2.4. Add equivalent volume of RoosterNourish™-MSC(-XF) to each vessel to stop the TrypLE activity.
2.5. Record total volume and transfer 0.5 mL of cells into microcentrifuge tubes for cell counts.

Total Volume of Cell Suspension (=A)

2.6. Count cells with a cell counting device, performing a dilution if required to get within its acceptable range:

Raw Data		Adjusted Data	
Dilution Factor (=B)	Viable Cell Concentration (=C)	Cell Concentration (D)=B*C	Total Cells at Harvest (E)=D*A

2.7. Transfer the cell suspension into a centrifuge tube.
2.8. Centrifuge at 350 x g for 6 min.

3. Formulation

Note: Cryopreservation leads to processing loss upon thaw, and RoosterBio recommends targeting >20% overfill to ensure target recovery.

3.1. Calculate volume of CS5 resuspension (recommended cryopreservation concentration range: 1-20M viable cells/mL):

Recorded Data		Calculated Data	
Viable Cell Concentration (=C)	Total Volume of Cell Suspension (=A)	Target Cryopreserved Concentration	Volume of CS5 (G)=A*C/F

3.2. Remove conical from centrifuge and aspirate the supernatant without disturbing the pellet.
3.3. Gently resuspend the cell pellet in CryoStor5 and mix until homogenous cell solution is obtained.
3.4. Aseptically aliquot cells into cryovials.
3.5. Tighten the cap and place them into freezing container(s) or controlled rate freezer within 45-90 minutes of resuspension.
3.6. Freeze at -1°C/min using Mr. Frosty isopropanol, CoolCell freezing container (Biocision), or controlled rate freezer until reaching -80°C.
3.7. Within 24 hours of freezing to -80° transfer into liquid nitrogen(vapor phase).