Thawing Human Mononuclear Cells

CMCI (Center for molecular and Cellular Intervention)
University Medical Center Utrecht

Written By

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Date</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mark Klein</td>
<td>Lab manager</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conformation

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
<th>Date</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof.dr. A.B.J. Prakken</td>
<td>Co-Chair CMCI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Changes from last version

<table>
<thead>
<tr>
<th>Date of version</th>
<th>Paragraphs</th>
<th>Date of version</th>
<th>Paragraphs</th>
</tr>
</thead>
</table>
### Thawing Human Mononuclear Cells

| Content |
|-----------------|-----------------|
| 1. Subject | 2. Application |
| 3. Definitions and Abbreviations | 4. Principle |
| 5. Safety precautions | 6. Reagents |
| 6.1 Chemicals | 6.2 Basic Culture Medium |
| 6.3 Wash Medium | 7. Equipment and Accessories tools |
| 7.1 Equipment | 7.2 Accessories |
| 8. Samples | 9. Procedure |
| Sample Processing | 10. Processing of the Results |
| 10.1 Calculation of the counted cells | 11. Documentation |
| 12. Accuracies and Precision | 13. Quality Control |
| 16. Literature |
Thawing Human Mononuclear Cells

1. **Subject**
   This Standard Operation Procedure (SOP) describes a method to thaw mononuclear cells from blood and synovial fluid.

2. **Application**

3. **Definitions and Abbreviations**
   - FBS = foetal bovine serum (≈ foetal calf serum)
   - RT = room temperature
   - P/S = Penicillin-Streptomycin
   - Glu = L-glutamine
   - v/v = volume/volume
   - rpm = rotations per minute
   - ml = milliliter
   - DMSO = Dimethyl Sulfoxide

4. **Principle**
   The thawing procedure should proceed without delay to secure a high viability of the cells. Thaw the cells in a beaker with warm water to prevent crystallization until a small bit of ice remains in the vial. To prevent a difference in osmotic pressure the DMSO concentration should be slowly diminished.

5. **Safety precautions**
   Treat every sample containing human material such as AB serum and the lymphocyte samples as infectious material. Wear disposable gloves.

**NOTE:** Be sure to wear a full-face shield during the thawing procedure because cryovials containing liquid nitrogen have been known to explode

- Suma-Tab contains sodium-di-chloro-isocynate Xn, Harmful
- Trypan blue (0.4%) T, Toxic
- Dry-Ice Xn, Harmful
- Ethanol F, Flammable
6. Reagents

6.1 Chemicals

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Formula</th>
<th>Supplier</th>
<th>order number</th>
<th>Store at (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI 1640</td>
<td>-</td>
<td>Invitrogen</td>
<td>52400-025</td>
<td>4</td>
</tr>
<tr>
<td>L-glutamine</td>
<td>-</td>
<td>Invitrogen</td>
<td>25030-024</td>
<td>-20</td>
</tr>
<tr>
<td>Penicillin-Streptomycin</td>
<td>-</td>
<td>Invitrogen</td>
<td>15140-122</td>
<td>-20</td>
</tr>
<tr>
<td>Foetal Bovine Serum</td>
<td>-</td>
<td>Invitrogen</td>
<td>10270-106</td>
<td>-20</td>
</tr>
<tr>
<td>Trypan Blue (0.4%)</td>
<td>C$<em>{34}$H$</em>{52}$N$<em>{6}$O$</em>{14}$S$<em>{4}$Na$</em>{6}$</td>
<td>Invitrogen</td>
<td>15250-061</td>
<td>RT</td>
</tr>
<tr>
<td>Ethanol</td>
<td>CH$_3$OH</td>
<td>Pharmacy (WKZ)</td>
<td></td>
<td>RT</td>
</tr>
</tbody>
</table>

6.2 Basic Culture Medium

RPMI 1640 supplemented with 1% v/v P/S (= 5ml) and 1% v/v glu (=5ml)

Store at 4°C up to 1 month

6.3 Wash Medium

RPMI 1640 supplemented with 1 v/v% P/S (= 5ml), 1 v/v% glu (=5ml) and 2% v/v FBS (=10 ml)

FBS should be heat inactivated (1 hr at 56°C) and filtered through a 0.20 µm sterile filter using a 10 ml sterile syringe before usage. Store at 4°C up to 1 month.

7. Equipment and Accessories tools

7.1 Equipment

- Centrifuge Hettich Rotanta 46 (UMC# 99-000-2142)
- Reichert brightline hemacytometer (Hausser Scientific Company, Horsham PA, USA)
- Easypet pipet (Eppendorf, Germany, 4006173)
- Pipets 10-1000 µl (Gilson, The Hague, The Netherlands)
- Multistep pipet (Eppendorf, Germany)

7.2 Accessories

- 50 ml sterile polypropylene conical tubes (Falcon/ Becton Dickinson, Erembodegem, Belgium, 352070)
- Sterile 10 ml syringe (Becton Dickinson, Erembodegem, Belgium)
- 2 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357507)
- 5 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357543)
- 10 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357551)
- 25 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357525)
- Disposable gloves (Kimberly Clark, Zaventem, Belgium)
Thawing Human Mononuclear Cells
8. Samples

Sample Processing
All handlings of the sample should be done in a biohazard safety cabinet

9. Procedure
1. Retrieve cryovial containing the cells from the storage area (-80°C or liquid nitrogen tank) and place on dry ice to minimize thawing
2. Spray the vials with alcohol and then wipe them dry
3. Thaw the cells by placing bottom half of the cryovial in a beaker with warm water (±37°C), do not submerge completely
4. Keep the vial in water until a small piece of ice remains
5. Spray the vials with alcohol and then wipe them dry
6. Gently open the cryovial and remove the contents from each vial by using a pipet
7. Dispense the cells into a 50 ml centrifuge tube, which already contains approximately 20 ml of wash medium (or basic medium with at least 2% FCS)
8. After adding all the cells of one patient to the tube, wash by filling up to 50 ml with wash medium
9. Centrifuge 6 min at 1200 rpm at RT to pellet the cells
10. Aspirate medium and resuspend the cell pellet in 50 ml culture medium
11. Repeat step #9 and 10
12. Centrifuge 6 min at 1200 rpm at RT to pellet the cells
13. Resuspend the cells in 5-10 ml wash medium
14. Count the cells via trypan blue exclusion method (See protocol: “UCAN-U 0001”). Count using the Reichert hemacytometer
15. Adjust to desired cell concentration

10. Processing of the Results
Calculation of the counted cells
Number of cells per ml =
Counted cells per mm² (25 squares) * dilution (10) * 10.000 =
Counted cells * 10⁶

11. Documentation
Document each sample used at your notebook. Remove the used vial with cells from the cell storage

12. Accuracies and Precision
Accuracy is hard to describe. Cell counts should be in a range of 75% of the original frozen cell numbers. Furthermore if the number of dead cells are higher then 20% it is desirable to run the sample over a ficoll gradient again (see “UCAN-U 0001”)
Thawing Human Mononuclear Cells

13. Quality Control
   N/A

   Instruction manual centrifuge
   Instruction manual safety cabinet
   Instruction how to handle liquid nitrogen

15. Remarks
   Cryovials are submerged in liquid nitrogen. So when a vial is not properly closed it will fill partly with nitrogen. When the vials are placed directly in warm water without letting the nitrogen evaporate by removing the lid of the vial, the nitrogen will expand and the vial will explode!

16. Literature
   N/A

*** END ***