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CDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell Hematopoietic Progenitor Cells as described herein. You assume all risk in connection with your use of iCell Hematopoietic Progenitor Cells.

Conditions of Use

iCell Hematopoietic Progenitor Cells are for life science research use only and subject to the use restrictions contained in Appendix A. You are responsible for understanding and performing the protocols described within this guide. CDI does not guarantee any results you may achieve. These protocols are provided as CDI’s recommendations based on its use and experience with iCell Hematopoietic Progenitor Cells.

Origin

iCell Hematopoietic Progenitor Cells are manufactured in the United States of America.

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Revision History

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Before You Begin

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Hematopoietic Progenitor Cells Prototype User’s Guide before handling or using iCell Hematopoietic Progenitor Cells.
- iCell Hematopoietic Progenitor Cells are for life science research use only. See Appendix A for more information and other restrictions.
- A Safety Data Sheet (SDS) for dimethyl sulfoxide (DMSO), in which iCell Hematopoietic Progenitor Cells are frozen, is available online at www.cellulardynamics.com/lit/ or on request from Cellular Dynamics International. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle iCell Hematopoietic Progenitor Cells.
**Chapter 1. Introduction**

Cellular Dynamics International's (CDI) iCell Hematopoietic Progenitor Cells are a highly pure population of human multipotent hematopoietic progenitor cells (HPCs) derived from induced pluripotent stem (iPS) cells using CDI’s proprietary differentiation and purification protocols. iCell Hematopoietic Progenitor Cells express the glycoprotein CD34 on the cell surface and are capable of generating in vitro blood cells of different lineages. As iCell Hematopoietic Progenitor Cells are maintained in culture and differentiated into hematopoietic lineages, the levels of CD34 expression decrease, and the expression of lineage specific markers is acquired. These cells provide a reliable source of human HPCs suitable for use in targeted drug discovery, toxicity testing, and other life science research.

When thawed and plated as recommended in this User’s Guide, iCell Hematopoietic Progenitor Cells retain their proliferative capacity and broad developmental potential. A supplementation of a commercially available HPC Culture Medium with selected combinations of cytokines and growth factors can drive the differentiation of iCell Hematopoietic Progenitor Cells into multiple lineages of the hematopoietic system including erythrocytes, granulocytes, and monocytes/macrophages. Given the transient nature of self-renewal and multipotency, iCell Hematopoietic Progenitor Cells should be used in proliferation and differentiation assays immediately after thawing.
### Components Supplied by Cellular Dynamics

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<thead>
<tr>
<th>Item</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCell Hematopoietic Progenitor Cells Prototype¹</td>
<td>HPC-301-020-001-PT</td>
</tr>
<tr>
<td>iCell Hematopoietic Progenitor Cells Prototype User’s Guide¹</td>
<td></td>
</tr>
<tr>
<td>Certificate of Testing²</td>
<td></td>
</tr>
<tr>
<td>Certificate of Origin If required for shipping purposes</td>
<td></td>
</tr>
</tbody>
</table>

¹ Safety Data Sheet and User’s Guide available online at www.cellulardynamics.com/lit/  
² Available online at www.cellulardynamics.com/cot/

### Required Equipment and Consumables

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor</th>
<th>Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equipment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37°C Water Bath</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Biological Safety Cabinet with UV Lamp</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Cell Culture Incubator</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Hemocytometer or Automated Cell Counter¹</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Liquid Nitrogen Storage Unit</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Pipettors</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Tabletop Centrifuge</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Tube Shaker</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td><strong>Consumables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 ml and 50 ml Centrifuge Tubes</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>D-PBS</td>
<td>Life Technologies</td>
<td>14190</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>FBS</td>
<td>Hyclone</td>
<td>SH30396.03</td>
</tr>
<tr>
<td>HPC Culture Medium²</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>IMDM</td>
<td>Life Technologies</td>
<td>12440</td>
</tr>
<tr>
<td>PES Filter Unit, 0.2 μm, 150 ml</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Pipettes</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Poly(2-hydroxyethyl methacrylate) (Poly-HEMA)</td>
<td>Sigma</td>
<td>P3932</td>
</tr>
<tr>
<td>Sodium Hydroxide (NaOH)</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Sterile Tissue Culture Grade Distilled Water</td>
<td>Multiple Vendors</td>
<td></td>
</tr>
<tr>
<td>Ultra Low Attachment Cell Culture Vessels</td>
<td>Corning</td>
<td>3815 (T25 Flask)</td>
</tr>
<tr>
<td>Untreated Cell Culture Vessels</td>
<td>Corning</td>
<td>431463 (T25 Flask)</td>
</tr>
</tbody>
</table>

¹ Ensure the automated cell counter is appropriately calibrated before use.  
² Recommended HPC Culture Medium include StemPro-34 SFM (Life Technologies, Cat. No. 10640-019, prepared according to the manufacturer’s instructions) or StemSpan SFEM (Stem Cell Technologies, Cat. No. 09650). HPC Culture Medium should be supplemented with hematopoietic growth factors and cytokines as required for intended use.
Technical Support and Training

CDI’s Technical Support Scientists have the necessary laboratory and analytical experience to respond to your inquiries. In addition, in-lab training may be available upon request.

**Telephone**
(877) 320-6688 (US toll-free) / (608) 310-5100 x5
Monday - Friday, 8:30 am - 5:00 pm US Central Time

**Fax**
(608) 310-5101

**Email**
support@cellulardynamics.com
Upon receipt, immediately transfer to LN₂ storage!

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Applications

Experimental Utilization (native biology, drug discovery, disease modeling, functionality, etc.)
Chapter 2. Handling and Storage

iCell Hematopoietic Progenitor Cells are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing iCell Hematopoietic Progenitor Cells to the vapor phase of a liquid nitrogen storage dewar. CDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.

It is critical to maintain cryopreserved iCell Hematopoietic Progenitor Cells at a stable temperature. Minimize exposure of cryopreserved iCell Hematopoietic Progenitor Cells to ambient temperature when transferring vials to liquid nitrogen storage.
Chapter 3. Preparing Cell Culture Surfaces

iCell Hematopoietic Progenitor Cells will function optimally in the following cell culture vessels:

- Ultra Low Attachment cell culture vessels
- Freshly coated cell culture vessels with poly-HEMA as described below

Preparing the Poly-HEMA Cell Culture Vessel

The following procedure details coating eight untreated T25 flasks. Scale volumes appropriately for other vessel formats.

1. Prepare a 95% ethanol solution by mixing the following components in a 50 ml centrifuge tube at the volumes specified:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume/40 ml</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol, 100%</td>
<td>38 ml</td>
<td>95%</td>
</tr>
<tr>
<td>NaOH, 1M</td>
<td>400 µl</td>
<td>10 mM</td>
</tr>
<tr>
<td>Sterile Tissue Culture Grade Distilled Water</td>
<td>1.6 ml</td>
<td>N/A</td>
</tr>
</tbody>
</table>

2. Add 4 g of poly-HEMA to the ethanol solution and immediately invert the centrifuge tube to prevent clumping.

3. Shake the tube at room temperature overnight.

4. Add 5 ml of poly-HEMA solution to each flask and rotate until all sides are coated, avoiding the cap.

5. Aspirate poly-HEMA solution from the flasks and let dry with the caps off in a biological safety cabinet overnight. It is recommended to aspirate the flasks 2 times to ensure complete removal of the poly-HEMA solution.

6. Rinse each flask with 5 ml of D-PBS before plating iCell Hematopoietic Progenitor Cells.

   Note: If necessary, store the flasks at 4°C for up to 1 week.
Chapter 4. Preparing the Thawing Medium

iCell Hematopoietic Progenitor Cells thawing requires preparation of the Thawing Medium. Prepare using sterile technique and store as follows:

1. Mix the following components at the volumes specified:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume/100 ml</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMDM</td>
<td>90 ml</td>
<td>90%</td>
</tr>
<tr>
<td>FBS</td>
<td>10 ml</td>
<td>10%</td>
</tr>
</tbody>
</table>

2. Filter the Thawing Medium using a 150 ml, 0.2 µm PES filter unit.
3. Store the Thawing Medium at 4°C, protected from light, for up to 1 month.
Chapter 5. Thawing iCell Hematopoietic Progenitor Cells

Maintain iCell Hematopoietic Progenitor Cells in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal iCell Hematopoietic Progenitor Cells viability and performance.

*Note:* Thaw no more than 1 vial at one time.

1. Equilibrate the Thawing Medium and HPC Culture Medium at room temperature for 2 - 4 hours before thawing iCell Hematopoietic Progenitor Cells.

2. Remove the frozen iCell Hematopoietic Progenitor Cells cryovial from the liquid nitrogen storage tank.

   *Note:* If necessary, place cryovials on dry ice for up to 10 minutes before thawing.

3. Immerse the cryovial in a 37°C water bath (avoid submerging the cap) and gently swirl for 2 minutes.

4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.

5. Gently transfer the iCell Hematopoietic Progenitor Cells cryovial contents to a 50 ml centrifuge tube using a 1 ml pipettor.

   *Note:* Use of a 50 ml centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase viability.

6. Rinse the empty iCell Hematopoietic Progenitor Cells cryovial with 1 ml of room temperature Thawing Medium to recover any residual cells from the vial. Transfer the 1 ml of medium rinse from the cryovial drop-wise (~1 drop/second) to the 50 ml centrifuge tube containing the iCell Hematopoietic Progenitor Cells suspension. Gently swirl the tube while adding the medium to mix the solution completely and minimize the osmotic shock on the thawed cells.

   *Drop-wise addition of the Thawing Medium to the cell suspension is critical to minimize osmotic shock and ensure maximum viability of the cells.*

7. Slowly add 8 ml of room temperature Thawing Medium to the 50 ml centrifuge tube (~1 - 2 drops/second). Gently swirl the centrifuge tube while adding the medium.

   *It is critical to add the 8 ml of Thawing Medium slowly to ensure maximum viability of the cells.*

8. Centrifuge the cell suspension at 300 x g for 5 minutes at room temperature.

9. Aspirate the supernatant from the 50 ml centrifuge tube being careful not to disturb the cell pellet.

10. Resuspend iCell Hematopoietic Progenitor Cells in 5 ml of HPC Culture Medium.
Chapter 6. Plating iCell Hematopoietic Progenitor Cells

Use iCell Hematopoietic Progenitor Cells in proliferation or differentiation assays immediately after thawing. Supplement the HPC Culture Medium with the appropriate cytokines and growth factors combinations to drive proliferation or differentiation into the lineage of choice.

1. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.

2. Dilute the cell suspension using room temperature HPC Culture Medium to obtain a desired cell plating density.

3. Culture iCell Hematopoietic Progenitor Cells in a cell culture incubator at 37°C, 5% CO₂.

Expected Cell Density

iCell Hematopoietic Progenitor Cells can be plated at various densities to accommodate different application needs. The thawing procedure will result in a viable cell concentration of approximately $2 \times 10^5$ cells/ml in 5 ml of total volume per vial. Figure 1 shows the expected density that can be obtained by plating $10 \times 10^5$ cells in a T25 flask.

Figure 1: iCell Hematopoietic Progenitor Cells at 24 Hours Post-plating

*These images show the expected morphology of iCell Hematopoietic Progenitor Cells.*
Appendices

Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License

A. OWNERSHIP. The Products are covered by pending patents and patents: cellulardynamics.com/about-us/patents/. Customer has a limited license to use the Products for internal research purposes for the sole benefit of the Customer, subject to the use restrictions and third party licenses included in subsection B of this Appendix A. Customer acknowledges and agrees that the receipt or purchase of the Products by Customer shall not be construed as a transfer of any title or the grant of any rights in or to the intellectual property embodied in the Products owned or licensed by Cellular Dynamics. In particular, no right or license to make, have made, offer to sell, or sell the Products, to modify or reproduce the Product or any part thereof, or to use the Products in combination with any other product(s), except product(s) provided or expressly licensed to Customer by Cellular Dynamics for such use, is implied or conveyed by the sale or transfer of Products to Customer.

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C. DATA. Customer agrees that if described on Customer's product quotation from Cellular Dynamics it will provide data and information as described therein to Cellular Dynamics regarding Customer's use of the Products.

Appendix B. Product Provided “AS IS”

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Appendix C. Limited Liability

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