CloneR™
Defined supplement for single-cell cloning of human ES and iPS cells
Catalog #05888 10 mL
Catalog #05889 5 x 10 mL

Product Description
CloneR™ is a defined, serum-free TeSR™ supplement designed to increase the cloning efficiency and single-cell survival of human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. CloneR™ enables the robust generation of clonal cell lines without single-cell adaptation, thus minimizing the risk of acquiring genetic abnormalities.

Properties
Storage: Store at -20°C.
Shelf Life: Stable for 2 years from date of manufacture (MFG) on label.

Materials Required but Not Included

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitronectin XF™</td>
<td>07180</td>
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<tr>
<td>OR</td>
<td></td>
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<tr>
<td>CellAdhere™ Laminin-521</td>
<td>77003</td>
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<td>OR</td>
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<tr>
<td>Corning® Matrigel® hESC-Qualified Matrix</td>
<td>354277</td>
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<tr>
<td>CellAdhere™ Dilution Buffer</td>
<td>07183</td>
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<tr>
<td>OR</td>
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<tr>
<td>DMEM/F-12 with 15 mM HEPES</td>
<td>36254</td>
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<tr>
<td>Enzymatic dissociation reagent(e.g., ACCUTASE™)</td>
<td>e.g. 07920</td>
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<tr>
<td>mTeSR™</td>
<td>85850</td>
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<tr>
<td>OR</td>
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<tr>
<td>mTeSR™ Plus</td>
<td>100-0276</td>
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<td>OR</td>
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<tr>
<td>TeSR™-E8™</td>
<td>05990</td>
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<tr>
<td>D-PBS (Without Ca++ or Mg++)</td>
<td>37350</td>
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</tbody>
</table>

Preparation of Reagents and Materials
Coating Cultureware with Cloning Matrix
Coat cultureware with cloning matrix; use Vitronectin XF™, CellAdhere™ Laminin-521, or Corning® Matrigel® hESC-Qualified Matrix.
NOTE: For Vitronectin XF™, use non-tissue culture-treated cultureware; for the other matrices, use tissue culture-treated cultureware.
NOTE: If using CellAdhere™ Laminin-521, coat cultureware the day before cloning, as overnight incubation is required.
1. Thaw cloning matrix according to the applicable Product Information Sheet (PIS) or manufacturer’s instructions.
2. Dilute cloning matrix according to Table 1.
Table 1. Dilution and Incubation of Cloning Matrix

<table>
<thead>
<tr>
<th>CLONING MATRIX</th>
<th>DILUENT</th>
<th>FINAL CONCENTRATION OF CLONING MATRIX</th>
<th>INCUBATION CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitronectin XF™</td>
<td>CellAdhere™ Dilution Buffer</td>
<td>10 µg/mL</td>
<td>Room temperature (15 - 25°C) for 1 hour</td>
</tr>
<tr>
<td>CellAdhere™ Laminin-521</td>
<td>CellAdhere™ Dilution Buffer</td>
<td>10 µg/mL</td>
<td>2 - 8°C overnight</td>
</tr>
<tr>
<td>Corning® Matrigel®</td>
<td>DMEM/F-12 with 15 mM HEPES</td>
<td>Refer to manufacturer’s instructions for reconstitution</td>
<td>Room temperature (15 - 25°C) for 1 hour</td>
</tr>
</tbody>
</table>

3. Add diluted cloning matrix to cultureware as follows:
   - **10 cm dish**: 6 mL
   - **96-well plate**: 50 µL/well
   NOTE: For other cultureware, use 100 µL/cm².

4. Tilt plate back and forth to distribute matrix solution evenly.
5. Incubate cultureware as indicated in Table 1.

Cloning Medium
The following example is for preparing 25 mL of cloning medium. If preparing other volumes, adjust accordingly.

1. Thaw CloneR™ at room temperature (15 - 25°C).
   NOTE: If not used immediately, store at 2 - 8°C. Do not exceed the shelf life of the supplement. Alternatively, aliquot and store at -20°C. Once aliquots are thawed, use immediately. Do not re-freeze.
2. Prepare complete mTeSR™1, mTeSR™ Plus, or TeSR™-E8™.
   NOTE: For complete instructions for media preparation, refer to the applicable PIS.
3. Add 2.5 mL of CloneR™ to 22.5 mL of complete mTeSR™1, mTeSR™ Plus, or TeSR™-E8™. Mix thoroughly.
   NOTE: If not used immediately, store cloning medium at 2 - 8°C for up to 1 week.

Directions for Use
Use ES or iPS cells cultured in mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ at day 6 - 8.
Please read the entire protocol before proceeding.
Use sterile technique when performing the protocols below.

A. PREPARING CULTUREWARE FOR CLONING
1. Aspirate cloning matrix from coated plates (see Preparation of Reagents and Materials).
2. Add cloning medium to cultureware as follows (initial seed volume):
   - **10 cm dish**: 8 mL
   - **96-well plate**: 100 µL/well
   NOTE: For other cultureware, use 150 µL/cm².

B. PREPARING A SINGLE-CELL SUSPENSION
1. Remove ES/iPS cell culture from incubator.
2. Under the microscope, mark regions of differentiation using a marker pen.
3. Remove regions of differentiation by aspiration. Rinse with D-PBS (Without Ca++ or Mg++) and aspirate.
4. Add enzymatic dissociation reagent (e.g. ACCUTASE™) at room temperature (15 - 25°C) at 1 mL per 10 cm² surface area. Incubate at 37°C for 5 - 8 minutes.
   NOTE: Optimal dissociation time may be cell line-dependent.
5. Rinse cells from cultureware by pipetting the dissociation reagent up and down, dispensing onto the colonies and breaking them up.
6. Dilute cell suspension 1 in 4 by adding to a conical tube (e.g. Catalog #38009) containing complete mTeSR™1, mTeSR™ Plus, or TeSR™-E8™.

7. Create a single-cell suspension by flicking the tube 3 - 5 times. Count cells using a hemocytometer or other cell counting method. Proceed to section C.

**C. PLATING CELLS (Day 0)**

**Plating at clonal density**

1. Dilute the cell suspension to 1 cell/µL in cloning medium (e.g. add 5000 cells and make up the volume to 5 mL with cloning medium).
2. Add desired number of cells to the cultureware prepared in section A.
   
   **NOTE:** The dissociation reagent should be sufficiently diluted so that it contains less than 1% final volume when added to the cultureware. Swirl the plate and rock back-and-forth 4 - 5 times to distribute cells evenly. Incubate at 37°C for 2 days. Proceed to section D.

**OR**

**Single-cell sorting (96-well plates)**

1. Centrifuge the single-cell suspension from section B at 300 x \(g\) for 5 minutes. Aspirate supernatant and resuspend cells at 1 x 10^6 cells/mL in cloning medium.
2. Pass the single-cell suspension from section B through a 37 µm Reversible Strainer (e.g. Catalog #27215/27250) to remove any large clumps.
3. Sort cells into individual wells of the plate prepared in section A using a fluorescence-activated cell sorter (FACS) (as low as 1 cell/well).
4. Incubate at 37°C for 2 days. Proceed to section D.

**D. FEEDING CELLS**

1. **Day 2:** Perform a full-medium change with cloning medium. Incubate at 37°C for 48 hours.
2. **Day 4+:** Perform a full-medium change with mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ (without CloneR™) daily until colonies are ready to be picked.