Matrigel Plate Coating

Brigitte Arduini, version 1, 2013-Jan-23
Modified from Scott Noggle, 2007-Sep-28

Matrigel (BD Biosciences), Geltrex (Life Technologies) and comparable substrates from other vendors are composed of extracellular matrix proteins derived from Engelbreth-Holm-Swarm (EHS) mouse tumor cells. The predominant component is laminin, but fibronectin, collagen and other proteins are also present. Not all substrates offered are specifically qualified for hESC maintenance.

Materials:

<table>
<thead>
<tr>
<th>Item</th>
<th>Vendor</th>
<th>Catalog No.</th>
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<tbody>
<tr>
<td>Matrigel, LDEV-free hESC-qualified matrix (5 ml)</td>
<td>VWR (BD Biosciences)</td>
<td>BD354277</td>
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<tr>
<td>DMEM/F12</td>
<td>Life Technologies</td>
<td>11330-032</td>
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<td>Cryovials, internal threads</td>
<td>VWR (Nunc)</td>
<td>66021-986</td>
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<td>Pipette tips, aerosol barrier</td>
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<td>Serological pipets, 10 ml</td>
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<td>15ml or 50ml Conical tube</td>
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All materials that will come in contact with Matrigel during plating must be cold to prevent premature gelling of the matrix. It may be convenient to store pipette tips at -20°C specifically for aliquoting Matrigel.

Matrigel Aliquots

Each Matrigel lot is provided with dilution instructions rather than a specific concentration.

1. Thaw 5ml vial of Matrigel overnight at 4°C.
2. Pre-chill cryovials and pipette tips.
3. Aliquot desired volume to cryovials according to Matrigel lot specifications (typically 300-500µl for later dilution to 25ml).
4. Freeze at -80°C for up to 6 months.

Matrigel Coating

1. Thaw one aliquot of Matrigel at 4°C. This may be done over several hours or overnight.
2. Pre-chill conical tube for dilution.
3. Culture vessels to be coated are pre-chilled by placing them on a metal incubator tray seated in a rectangular ice bucket.
4. With chilled pipette tips, add aliquot of Matrigel to appropriate volume of cold DMEM/F12 (typically ~400µl Matrigel in 25ml DMEM/F12).
5. Chill a 10ml serological pipet by opening the top of the sleeve slightly and dipping the pipet (still in the sleeve) into liquid nitrogen.
6. Immediately distribute the diluted Matrigel to waiting tissue culture vessels.
7. Store tissue culture vessels at 4°C until needed.
To use Matrigel-coated plates:

1. Before plating hESCs, place Matrigel dishes in the incubator for at least 20 minutes, but no more than one hour. Longer incubation times lead to excess matrix and three-dimensional growth of hESCs.
2. Check formation of matrix under the microscope prior to plating cells.
3. Aspirate matrigel, removing as much liquid as possible without scraping the bottom of the dish. It is not necessary to rinse Matrigel-coated dishes.
4. Plate hESCs in desired maintenance medium.