CloneR™

Defined supplement for single-cell cloning of human ES and iPS cells

Catalog #05888 10 mL
Catalog #05889 5 x 10 mL

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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

PRODUCT NAME	CATALOG #
Vitronectin XF™ OR CellAdhere™ Laminin-521 OR Corning® Matrigel® hESC-Qualified Matrix	07180 OR 77003 OR 07181
CellAdhere™ Dilution Buffer OR DMEM/F-12 with 15 mM HEPES	07183 OR 36254
Enzymatic dissociation reagent (e.g. ACCUTASE™)	e.g. 07920
mTeSR™1 OR mTeSR™ Plus OR TeSR™-E8™	85850 OR 05825 OR 05990
D-PBS (Without Ca++ or Mg++)	37350

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).
2. Dilute cloning matrix according to Table 1.

Table 1. Dilution and Incubation of Cloning Matrix

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

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/cm^2.
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/cm^2.
3. Incubate at 37°C for 1 hour prior to seeding cells.

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 cm^2 surface area. Incubate at 37°C for 5 - 8 minutes.

   NOTE: 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. Falcon® Conical Tube, 15 mL, 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. Centrifuge the single-cell suspension from section B at 300 x *g* for 5 minutes. Aspirate supernatant and resuspend cells in cloning medium.
2. Add desired number of cells to the cultureware containing warm cloning medium from section A.
3. 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 containing warm cloning medium (from 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 24 hours.
2. **Day 3**: Add cloning medium (25% of initial seed volume). Incubate at 37°C for 24 hours.
3. **Day 4 - 10/14**: Perform a full medium change with mTeSR™1, mTeSR™ Plus, or TeSR™-E8™ (without CloneR™) daily until colonies are ready to be picked.