eTeSR™

Stabilized, feeder-free culture medium optimized for single-cell passaging of human pluripotent stem cells

Catalog #100-1215 1 Kit

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Product Description

eTeSR™ is a stabilized, feeder-free cell culture medium optimized for the single-cell maintenance and expansion of human pluripotent stem cells (hPSCs). eTeSR™ builds upon previous TeSR™ formulations,[1,2] the most widely published feeder-free cell culture media for hPSCs.

eTeSR™ has been specifically developed to reduce the cellular stress associated with passaging hPSCs as single cells, which typically involves shorter passaging schedules at high culture densities. To cope with the increased metabolic demand and cell stress associated with single-cell passaging, eTeSR™ has improved buffering capacity and optimized metabolites to produce high-quality hPSCs with enhanced genetic stability. With stabilized key components, including fibroblast growth factor 2 (FGF2; also known as basic FGF [bFGF]), eTeSR™ is compatible with both daily and restricted feeding schedules while maintaining high cell quality and equivalent performance.

eTeSR™ is compatible with a variety of cell culture matrices for long-term routine culture, including Corning® Matrigel® hESC-Qualified Matrix (Corning Catalog #354277) and CellAdhere™ Laminin-521 (Catalog #200-0117).

Each lot of eTeSR™ 10X Supplement is quality-tested in a culture assay using hPSCs.

Product Information

The following components are sold as a complete kit (Catalog #100-1215) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
eTeSR™ Basal Medium	100-1216	450 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
eTeSR™ 10X Supplement	100-1217	50 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
CloneR™2 OR Y-27632 (Dihydrochloride)	100-0691 OR 72302
Conical tubes, 15 mL	e.g. 38009
Corning® Matrigel® hESC-Qualified Matrix OR CellAdhere™ Laminin-521	Corning 354277 OR 200-0117
D-PBS (Without Ca++ and Mg++)	37350
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
Trypan Blue	07050
TrypLE™ Express Enzyme OR ACCUTASE®	Thermo Fisher 12605010 OR 07920

Preparation of Reagents and Materials

Use sterile technique to prepare complete eTeSR™ medium (eTeSR™ Basal Medium + eTeSR™ 10X Supplement). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

A. COMPLETE eTeSR™ MEDIUM

1. Thaw eTeSR™ 10X Supplement at room temperature (15 - 25°C) or overnight at 2 - 8°C. **Do not thaw in a 37°C water bath.** Mix thoroughly by swirling or gently inverting the bottle. Do not shake.

   NOTE: eTeSR™ 10X Supplement may appear slightly cloudy after thawing. This will not affect performance.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C for up to 3 months. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.
2. Add 50 mL of eTeSR™ 10X Supplement to 450 mL of eTeSR™ Basal Medium. Mix thoroughly by swirling or gently inverting the bottle. Do not shake. Warm to room temperature before use (do not warm complete eTeSR™ medium in a 37°C water bath).

   NOTE: If not used immediately, store complete eTeSR™ medium at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C for up to 3 months. Do not exceed the shelf life of the individual components. After thawing the aliquoted complete medium, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-freeze.

If prepared aseptically, complete eTeSR™ medium is ready for use. If desired, the medium can be filtered using a 0.2 - 0.22 µm low protein binding polyethersulfone (PES) filter unit (e.g. Fisher 09-741-04 [0.2 µm, 250 mL]; Fisher SCGP00525 [0.22 µm, 50 mL]).

B. COATING CULTUREWARE WITH CORNING® MATRIGEL® OR CELLADHERE™ LAMININ-521

For complete instructions on coating cultureware with Corning® Matrigel® hESC-Qualified Matrix or CellAdhere™ Laminin-521, refer to the Technical Manual: Expansion of Human Pluripotent Stem Cells in eTeSR™ (Document #10000019963), available at www.stemcell.com or contact us to request a copy.

NOTE: Use tissue culture-treated cultureware (e.g. Falcon® 6-Well Flat-Bottom Plate, Tissue CultureTreated, Catalog #38016) with Corning® Matrigel® or CellAdhere™ Laminin-521.

Directions for Use

For complete instructions on how to maintain hPSCs in eTeSR™, refer to the Technical Manual: Expansion of Human Pluripotent Stem Cells in eTeSR™.

Harvesting and Seeding hPSCs as Single Cells

The following instructions are for preparing a single-cell suspension of hPSCs in one well of a 6-well plate using TrypLE™ Express Enzyme. If using other cultureware, adjust volumes accordingly. hPSCs are ready to be passaged when the culture reaches approximately 70 - 95% confluency. Visually check the quality of the cultures before passaging. The culture should have minimal (< 2%) spontaneous differentiation and display characteristic hPSC morphology (i.e. high nuclear-to-cytoplasm ratio and prominent nucleoli).

NOTE: If desired, hPSCs may be dissociated with ACCUTASE®. For complete instructions, refer to the Technical Manual: Expansion of Human Pluripotent Stem Cells in eTeSR™.

1. Warm complete eTeSR™ medium (Preparation section A), TrypLE™ Express Enzyme, and coated culture plate(s) (Preparation sections B & C) to room temperature (15 - 25°C).

   NOTE: Do not warm complete eTeSR™ medium in a 37°C water bath.
2. Prepare Single-Cell Plating Medium by adding the desired plating supplement to an appropriate volume of complete eTeSR™ medium as follows:
   - CloneR™2: Add at a 1 in 10 dilution (e.g. add 10 mL of CloneR™2 to 90 mL of complete eTeSR™ medium).
   - Y-27632 (Dihydrochloride): Add to a final concentration of 10 µM.
3. Wash the well to be passaged with 2 mL of D-PBS (Without Ca++ and Mg++).
4. Discard the D-PBS wash and add 0.5 mL of TrypLE™ Express Enzyme.
5. Incubate at 37°C and 5% CO_2 for 4 - 7 minutes.

   NOTE: The incubation time may vary depending on the cell line, matrix, or dissociation reagent used, as well as the culture confluency at the time of passaging. Four minutes of incubation at 37°C was found to be optimal for most cell lines maintained on Corning® Matrigel® at 70 - 95% confluency. A longer incubation time may be required when using CellAdhere™ Laminin 521 or performing automated passaging protocols.
6. Detach the cells from the surface of the well as follows:

   a. Add 1 mL of complete eTeSR™ medium (prepared in step 1) to the well.

b. Tilt the plate at approximately a 45° angle. Using a P1000, gently dispense the medium directly onto the well surface starting at the top and working down. Spray the well surface with the cell supsension an additional 1 - 2 times to detach any remaining cells.

NOTE: Avoid excessive or harsh trituration, as this may adversely impact cell viability. Dispense the wash with just enough force to detach the cells from the well surface. If the cells do not readily detach, a longer incubation time may be required.

c. Using the same P1000 pipette or a 2 mL serological pipette (e.g. Catalog #38002), transfer the single-cell suspension to a 15 mL conical tube containing an additional 1 - 4 mL of complete eTeSR™ medium.

7. Centrifuge the cell suspension at 300 x *g* for 5 minutes.
8. Remove the coating matrix from the culture plate(s) prepared in step 1 and immediately add 2 mL/well of Single-Cell Plating Medium.
9. Discard the supernatant and gently flick the tube 3 - 5 times to resuspend the cell pellet.
10. Add 1 - 2 mL of Single-Cell Plating Medium (prepared in step 2) to the cells without triturating.
11. Count cells using Trypan Blue and a Hausser Scientific™ Bright-Line Hemocytometer (or an automated cell counting method).
12. Seed the cells in the coated culture plate(s) prepared in step 1. Refer to table 1 for recommended seeding densities to achieve approximately 70 - 95% confluency for a day 4 or day 5 passaging schedule.

    NOTE: It is important to plate a range of cell densities to ensure that at least one well will reach 70 - 95% confluency on the desired day of passaging (i.e. 4 or 5 days after seeding).

    NOTE: Cell seeding densities are cell line-dependent and may need to be optimized for individual cell lines. Cultures grown in eTeSR™ may exhibit enhanced growth rates compared to those grown in other TeSR™ media. Therefore, users may need to reduce the seeding density as required when adapting cultures to eTeSR™.

Table 1. Recommended Seeding Densities for hPSC Cultures to Reach 70 - 95% Confluence in 4 or 5 Days

DAYS BEFORE hPSCs REACH CONFLUENCE	EXAMPLE NUMBER OF CELLS SEEDED PER WELL OF A 6-WELL PLATE (~10 cm^2)		
	LOW DENSITY	MEDIUM DENSITY	HIGH DENSITY
4 Days	2 x 10^4	5.5 x 10^4	9.5 x 10^4
5 Days	1.8 x 10^4	6.5 x 10^4	4 x 10^4

13. Move the plate in several quick, short, back-and-forth and side-to-side motions to distribute the hPSCs across the surface of the wells. Incubate at 37°C and 5% CO_2. Do not disturb the plate for 24 hours.
14. Remove Single-Cell Plating Medium from the wells and add 2 mL of complete eTeSR™ medium per well. Incubate at 37°C and 5% CO_2 for 24 hours.
15. Perform medium changes as needed using complete eTeSR™ medium and visually assess cultures to monitor growth until the cells reach ~70 - 95% confluence. The medium can be changed daily or every other day. To skip two consecutive days of feeding, add twice the volume of medium (e.g. 4 mL/well of a 6-well plate).

Feeding and Passaging Schedules

eTeSR™ accommodates a more flexible feeding schedule without affecting performance or cell quality. To determine a convenient schedule that suits your lab's routine, refer to Table 2 below. Any combination of feeding intervals can be used throughout a passage when following these guidelines.

Table 2. Flexible Feeding Intervals

FEEDING INTERVAL		
DAILY FEEDING	SKIP ONE DAY	SKIP TWO CONSECUTIVE DAYS
Standard feed volume (e.g. 2 mL per well of a 6-well plate)	Standard feed volume (e.g. 2 mL per well of a 6-well plate)	Double feed volume (e.g. 4 mL per well of a 6-well plate)

Single-cell hPSC cultures maintained in eTeSR™ should be passaged every 4 - 5 days once the cells reach 70 - 95% confluence. Passaging cultures on a Monday/Friday/Wednesday schedule allows for a weekend-free passaging protocol (see Table 3) that is compatible with the feeding schedules detailed in Table 2. We recommend performing a full-medium change 24 hours after passaging, as the prolonged presence of seeding supplements (e.g. CloneR™2 or Y-27632) in the culture may affect cell quality. Additionally, cultures grown in eTeSR™ may exhibit improved growth rates compared to those grown in other TeSR™ media.

Table 3. hPSC Maintenance Schedule with Minimal Weekend Work

WEEK 1	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
	Passage*	Feed	-	Feed	**Passage****	Feed	-
WEEK 2	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
	Feed	-	**Passage****	Feed	Double Feed	-	-

*Cells seeded to reach confluency in 4 days.
**Cells seeded to reach confluency in 5 days.

Assessment of hPSCs

The following antibodies can be used to characterize the undifferentiated state of hPSCs by flow cytometry or immunocytochemistry:

- Anti-Human SSEA-4 Antibody, Clone MC-813-70 (Catalog #60062), and
- Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R (Catalog #60064), and
- Anti-Human OCT4 (OCT3) Antibody, Clone 3A2A20 (Catalog #60093)

For complete flow cytometry protocols and antibodies that can be used, refer to the Technical Manual: Expansion of Human Pluripotent Stem Cells in eTeSR™, available at www.stemcell.com, or contact us to request a copy.

Related Products

For related products, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCworkflow, or contact us at techsupport@stemcell.com.

References

1. Ludwig TE et al. (2006) Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol 24(2): 185–7.
2. Ludwig TE et al. (2006) Feeder-independent culture of human embryonic stem cells. Nat Methods 3(8): 637–46.