gibco
by Thermo Fisher Scientific

Fusion Medium Kit (Prototype)

Description

Fusion Medium is a robust, serum albumin containing medium which supports the expansion of pluripotent stem cells (PSCs) without the need for daily feeding, allowing for weekend-free culture of PSCs. Fusion Medium provides optimum cell survival in single cell passaging applications and therefore supports critical applications, such as gene editing.

Product*	Catalog No.	Amount	Storage
Fusion Medium Kit (Prototype) contains:	**A32719**	**1 Kit**	
Fusion Basal Medium (Prototype)	A32711	450 mL	Store at 2°C to 8°C. Protect from light.
Fusion Supplement (10X) (Prototype)	A32712	50 mL	Store at –5°C to –20°C. Protect from light.

* Fusion Medium Kit (Prototype) is sold as a complete kit; individual components are not sold separately.

Product use

For Research Use Only. Not for use in diagnostic procedures. Product is a prototype and performance characteristics and shelf-life of this product have not been established.

Important information

- Thaw frozen Fusion Supplement (10X) at room temperature for ~2 hours or overnight at 2°C to 8°C to prepare complete medium. **Thawing of frozen supplement at 37°C is not recommended**.
- Following reconstitution, complete media can be aliquoted and stored at –5 to–20 °C. Alternatively, usage size aliquots of the supplement can be made and frozen at –5 to–20 °C. Avoid multiple freeze-thaw cycles.
- For complete transition to the Fusion Medium system, three passage adaptation is recommended.

Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture conditions

Media: Complete Fusion Medium

Culture type: Adherent

Compatible substrates:

- Single cell passaging in the presence of RevitaCell™ Supplement or ROCK Inhibitor or routine clump cell passaging:
  - Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix (Cat. No. A14133)
  - Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Cat. No. A14700)
- Single cell passaging in absence of RevitaCell™ Supplement or ROCK Inhibitor:
  - Recombinant Human Laminin-521 (rhLaminin-521) (Cat. No. A29248)

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Prepare complete Fusion Medium (500 mL)

1. Thaw the frozen Fusion Supplement (10X) at room temperature for ~2 hours or overnight at 2 to 8 °C. **Do not thaw the frozen supplement at 37°C.**
2. Mix the thawed supplement by gently inverting 3–5 times.
3. Aseptically transfer 50 mL of Fusion Supplement (10X) to the bottle of Fusion Basal Medium (450 mL fill). Gently invert the bottle several times to obtain 500 mL of homogenous complete medium.
4. Complete Fusion Medium can be stored at 2°C to 8°C for up to 2 weeks. Before use, warm complete medium required for that day at room temperature until it is no longer cool to the touch. Alternatively, an aliquot for use that day may be prewarmed in a 37°C waterbath until no longer cool to the touch. Avoid extended dwell times at 37°C.

Human PSC culture in Fusion Medium

- Split cultures when the first of the following occurs:
  (a) PSC colonies become too dense or too large;
  (b) PSC colonies show increased differentiation;
  (c) Colonies cover ~85% of the surface area of the culture vessel, usually every 3 to 5 days.
- The split ratio can vary, though it is generally between 1:2 and 1:4 for newly derived PSCs and between 1:3 and 1:12 for established cultures. Occasionally, cells may recover at a different rate and the split ratio will need to be adjusted.
- A general rule is to observe the last split ratio and adjust the ratio according to the appearance of the PSC colonies. If the cells look healthy and the colonies have enough space, split using the same ratio. If the colonies are overly dense and crowding, increase the ratio; if they are sparse, decrease the ratio.
- Newly derived PSC lines may contain a fair amount of differentiation through passage 4. It is not necessary to remove differentiated material prior to passaging. By propagating/splitting the cells the overall culture health should improve throughout the early passages.
- **Do not scrape the cells from the culture vessel during passaging.**

Recover frozen PSCs in complete Fusion Medium

1. Refer to the Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix and VTN-N user guides or Table 1 for recommended coating conditions.Pre-warm complete Fusion Medium.

   Note: If using pre-coated plates stored at 2 to 8°C, then prewarm Geltrex™ matrix or VTN-N-coated plates to room temperature.

2. Remove the vial of PSCs from liquid nitrogen storage and transfer it on dry ice to the tissue culture room.

3. Immerse the vial in a 37°C water bath without submerging the cap. Swirl the vial gently. When only an ice crystal remains, remove the vial from the water bath, spray the outside of it with 70% ethanol, and place it in the hood.

4. Transfer the thawed cells to a 15-mL conical tube and add 3 mL of complete Fusion Medium drop-wise to the cells. This reduces osmotic shock to the cells. While adding the medium, gently move the tube back and forth to mix the PSCs.

5. Rinse the vial with 1 mL of complete Fusion Medium and add to the 15 mL tube with cells.

6. Centrifuge the cells at 200 × g for 4 minutes, aspirate and discard the supernatant, and resuspend the cell pellet in 1 mL of complete Fusion Medium by gently pipetting the cells up and down a few times.

7. Slowly add the PSC suspension into pre-warmed Geltrex™ matrix or VTN-N-coated plate, plating ~100,000 viable cells per cm^2 of plate for conditions seeded in the absence of ROCK inhibitor.

   Optional: To improve efficiency of cell survival 24 hours post-thaw, inclusion of RevitaCell™ Supplement (Cat. No. A2644501) may be used at 1X final concentration (i.e., 10 µL per 1 mL of cell suspension) for the first 24 hours post-thaw to minimize apoptosis and necrosis. When using this supplement for recovery of your PSCs, lower cell seeding densities are required; plating at a viable cell density of ~20,000-40,000 viable cells/cm^2 will allow for recovery in three-four days post-thaw. **Do not include additional ROCK inhibitors such as Y-27632 or thiazovivin when using RevitaCell™ Supplement**.

8. Move the plate in several quick side-to-side motions to disperse the cells across the surface of the wells and place the plate gently into the 37°C, 5% CO_2 incubator.

9. Feed the PSCs the day after seeding followed by every-other-day thereafter. If the cells are to be left without feeding for two days (for example, over a weekend), then double the feed volume (refer to Figure 1 for typical flexible feeding schedule).

   Note: Cells should be passaged once reaching ~85% confluency to maintain optimum cell health of cultures.

Clump cell passage PSCs using Versene solution for routine culture

1. Pre-warm Fusion Medium, Geltrex™ matrix or VTN-N-coated culture vessels, and Versene solution to room temperature.

2. Aspirate the spent medium from the vessel containing PSCs and rinse the vessel once with DPBS no calcium, no magnesium (refer to Table 1 for recommended volume).

3. Add Versene solution to the side of the vessel containing PSCs (refer to Table 1). Swirl the vessel to coat the entire well surface.

4. Incubate the vessel at room temperature for 5 to 8 minutes or at 37°C for 4 to 5 minutes. When the cells start to separate and round up, and the colonies appear to have holes in them when viewed under a microscope, they are ready to be removed from the vessel.

   Note: Cells should not be incubated to the extent that the colonies float off the surface of the culture vessel.

5. Aspirate the Versene solution, and add pre-warmed complete Fusion Medium to the vessel (refer to Table 1).

6. Remove the cells from the well(s) by gently flushing medium over the surface of the well a few times and pipetting the colonies up and down. Avoid creating bubbles. Collect cells in a 15-mL conical tube. There may be obvious patches of cells that were not dislodged and left behind. Do not scrape the cells from the dish in an attempt to recover them.

   Note: Depending upon the cell line, work with no more than 1 to 3 wells at a time, and work quickly to remove cells after adding Fusion Medium to the well(s), which quickly neutralizes the initial effect of the Versene. Some lines re-adhere very rapidly after medium addition, and must be removed 1 well at a time. Others are slower to re-attach, and may be removed 3 wells at a time.

7. Add an appropriate volume of pre-warmed complete Fusion Medium to each well of a Geltrex™ matrix or VTN-N-coated plate so that each well contains the recommended volume of complete medium after the cell suspension has been added as per Table 1.

   Note: Step 7 can be completed prior to passaging the cells.

8. Move the vessel in several quick side-to-side motions to disperse the cells across the surface of the vessels. Place the vessel gently into the 37°C, 5% CO_2 incubator and incubate the cells overnight.

9. Feed the PSCs the day after passaging followed by every-other-day thereafter until the cells are approximately 85% confluent. If the cells are to be left without feeding for longer than 48 hours (for example, during a weekend), double the feed volume.

   Note: It is normal to see cell debris and small colonies after passage. Cells should be passaged once reaching ~85% confluency to maintain optimum cell health of cultures.

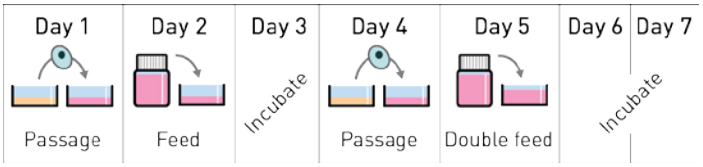

Figure 1 Typical weekly PSC culture workflow using Fusion Medium

Table 1 Reagent volumes for Clump Cell Passaging (in mL per well or per dish)

Culture vessel (~ surface area)	VTN-N or Geltrex™ solution*	DPBS (-/-) for wash	Versene solution	Complete medium
6-well (10 cm^2)	2 mL	2 mL	1 mL	2 mL
12-well (4 cm^2)	0.8 mL	1 mL	0.4 mL	1 mL
24-well (2 cm^2)	0.4 mL	0.5 mL	0.2 mL	0.5 mL
35-mm (10 cm^2)	2 mL	2 mL	1 mL	2 mL
60-mm (20 cm^2)	4 mL	4 mL	2 mL	4 mL
100-mm (60 cm^2)	12 mL	12 mL	12 mL	12 mL

*The optimal working concentration of Geltrex™ matrix and VTN-N is cell line dependent.

- We recommend using a final 1:200 dilution of Geltrex™ matrix in cold DMEM/F12 + GlutaMAX™ solution.

  Note: Thaw Geltrex™ matrix overnight and mix by gentle inversion. Do not allow Geltrex™ matrix to be exposed to room temperature, rather transfer on an ice bucket to the cell culture hood to minimize gelling. Geltrex™ matrix can be combined 1:1 with DMEM/F12 + GlutaMAX™ solution and divided into usage size aliquots and stored at –20°C until further use.

- For VTN-N, we recommend using a final coating concentration of 0.1–1.0 µg/cm^2 on the culture surface, depending on your cell line. The volumes listed above are based on a final coating concentration of 0.5 µg/cm^2, using a 1:200 VTN-N solution in DPBS no calcium, no magnesium.

  Note: Thaw VTN-N at room temperature and mix by gentle inversion. VTN-N can be divided into usage size aliquots and stored at –80°C until further use.

Single cell passage PSCs using TrypLE™ Select for High Throughput Screening (HTS) or gene editing applications

1. Pre-warm Fusion Medium, Geltrex™ matrix, VTN-N, or rhLaminin-521-coated culture vessels, and TrypLE Select solution to room temperature.

   Note: rhLaminin-521 is the recommended matrix for gene editing applications. We recommend using a final coating concentration 0.5–2 µg/cm^2. Dilute rhLaminin-521 in either DPBS, calcium, magnesium or DMEM/F12 + GlutaMAX™ solution or Fusion Basal Medium. Do not use complete Fusion Medium.

   Note: Thaw rhLaminin-521 at 2 to 8°C and mix by gentle inversion. Thawed rhLaminin-521 can be divided into usage size aliquots and stored at –20°C until expiration date or at 2°C to 8°C for up to 3 months.

2. Aspirate spent medium from the culture vessel.
3. Rinse the vessel once with recommended volume of DPBS no calcium, no magnesium (see Table 2).
4. Add recommended volume of prewarmed TrypLE™ Select (see Table 2).
5. Incubate the vessel at 37°C, 5% CO_2 for 5 minutes.

   Note: Avoid extended incubation of PSCs with dissociation reagent to minimize cellular toxicity.

6. Gently pipette the cells up and down 5–10 times to generate a single cell suspension.
7. Transfer the cell suspension to a conical tube containing the recommended volume of Fusion Medium to dilute the dissociation reagent (see Table 2).
8. Centrifuge the PSCs at 200 × g for 4 minutes.
9. Aspirate and discard the supernatant, flick the tube 3–5 times to loosen the pellet, and resuspend the cells by pipetting them up and down 5–10 times in the recommended volume of Fusion Medium (see Table 2).
10. Determine the viable cell density and percent viability using a Countess™ II Automated Cell Counter or similar automated or manual method.
11. Adjust the concentration of the cell suspension using Fusion Medium to achieve the cell seeding density recommended for your culture vessel (see Table 3).

    Note: Cell seeding densities are cell line dependent and thus may need to be optimized for your cell line.

12. Transfer the cell suspension to the pre-coated culture vessel.

    Note: If using Geltrex™ matrix or rhVTN-N then inclusion of 1X RevitaCell™ Supplement for the first 24 hours post-passage is recommended, whereas rhLaminin-521 does not require inclusion of RevitaCell™ Supplement.

13. Move the vessel in several quick side-to-side motions to disperse the cells across the surface of the vessel. Place the vessel gently into the 37°C, 5% CO_2 incubator and incubate the cells overnight.
14. Feed the PSCs the day after passaging followed by every-other-day thereafter. If the cells are to be left without feeding for longer than 48 hours (for example, during a weekend), double the feed volume.

    Note: It is normal to see cell debris and small colonies after passage

15. Cells should be passaged once reaching ~85% confluency to maintain optimum cell health of cultures.

Table 2 Reagent volumes for Single Cell Passaging (in mL per well or per dish)

Culture vessel (surface area)	Diluted Geltrex™, VTN-N, or rhLaminin-521	DPBS(-/-) for wash	TrypLE™ Select	Neutralization volume, Fusion Medium	Resuspension volume, Fusion Medium
6-well (10 cm^2)	2 mL	2 mL	1 mL	3 mL	2 mL
12-well (4 cm^2)	0.8 mL	1 mL	0.4 mL	1.2 mL	1 mL
24-well (2 cm^2)	0.4 mL	0.5 mL	0.2 mL	0.6 mL	0.5 mL
35-mm (10 cm^2)	2 mL	2 mL	1 mL	3 mL	2 mL
60-mm (20 cm^2)	4 mL	4 mL	2 mL	6 mL	4 mL
100-mm (60 cm^2)	12 mL	12 mL	6 mL	18 mL	12 mL

Table 3 Recommended single cell passaging cell seeding densities and volumes of medium for plating (per well or per dish)

Culture vessel (surface area)	Number of viable cells added*		Resuspension volume, Fusion Medium
	12,500 cells/cm^2	25,000 cells/cm^2	
6-well (10 cm^2)	125,000	250,000	2 mL
12-well (4 cm^2)	50,000	100,000	1 mL
24-well (2 cm^2)	25,000	50,000	0.5 mL
35-mm (10 cm^2)	125,000	250,000	2 mL
60-mm (20 cm^2)	250,000	500,000	4 mL
100-mm (60 cm^2)	750,000	1,500,000	12 mL

* Time to confluency is 4–5 days for a seeding density of 12,500 cells/cm^2 and 3–4 days for a seeding density of 25,000 cells/cm^2. Seeding densities may be cell line dependent and may require optimization for your lines.

Related products

Product	Cat. No.
Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	A14133
DMEM/F-12, GlutaMAX™ Supplement	10565
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
DPBS no calcium, no magnesium	14190
rhLaminin-521	A29248
DPBS, calcium, magnesium	14040
Versene Solution	15040
TrypLE™ Select Enzyme (1X), no phenol red	12563
RevitaCell™ Supplement	A26445
PSC Cryopreservation Kit	A26446

Explanation of symbols and warnings

				
Caution, consult accompanying documents	Temperature Limitation	Keep away from light	Use By:	Consult instructions for use
LOT	REF		STERILE A	Read SDS
Batch Code	Catalog number	Manufacturer	Sterilized using aseptic processing techniques	Read Safety Data Sheet

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