StemFlex™ Medium Kit

Catalog Number A3349401

Pub. No. MAN0016431 **Rev.** 2.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ StemFlex™ Medium supports the robust expansion of feeder-free pluripotent stem cells (PSCs) and is optimized to support novel applications including single cell passaging, gene editing and reprogramming. The StemFlex™ Medium unique formulation offers modern conveniences of a flexible feeding schedule (including weekend-free options) and also the ability to choose the matrix and passaging reagent that best suits specific applications.

Contents and storage

StemFlex™ Medium Kit[1], Cat. No. A3349401

Contents	Cat. No.	Amount	Storage	Shelf life[2]
StemFlex™ Basal Medium	A3349301	450 mL	Store at 2°C to 8°C. Protect from light.	12 months
StemFlex™ Supplement 10X[3]	A3349201	50 mL	Store at –5°C to –20°C. Protect from light.	

[1] StemFlex™ Medium Kit is sold as a complete kit; individual components are not sold separately.
[2] Shelf-Life duration is determined from Date of Manufacture.
[3] Store in a non-frost-free freezer at –5°C to –20°.

Culture conditions

Medium: StemFlex™ Medium

Culture type: Adherent

Recommended substrates:

- For clump cell passaging using Versene Solution or 500 µM EDTA we recommend Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix (Geltrex™ matrix) (Cat. No. A14133).
- For single cell passaging using TrypLE™ Select we recommend Recombinant Human Laminin-521 (rhLaminin-521) (Cat. No. A29248, A29249).
- Refer to FAQs for additional compatible matrices and combinations.

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 StemFlex™ Medium

1. Thaw the frozen StemFlex™ Supplement 10X at room temperature for ~2 hours or overnight at 2°C to 8°C.

   IMPORTANT! 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 StemFlex™ Supplement 10X to the bottle of StemFlex™ Basal Medium (450 mL fill).

   Gently invert the bottle several times to obtain 500 mL of homogenous complete medium.
4. Store complete StemFlex™ Medium at 2°C to 8°C for up to 2 weeks.
5. 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 pre-warmed in a 37°C waterbath until no longer cool to the touch. Avoid extended dwell times at 37°C.

   Following reconstitution, complete media can be aliquoted and stored at –5°C to –20 °C for up to 6 months. Alternatively, usage size aliquots of the supplement can be made and frozen at –5 to–20 °C for up to 6 months. Avoid multiple freeze-thaw cycles.

Procedural guidelines

Guidelines for culturing human PSCs in StemFlex™ 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 clump cell passaging using Versene Solution or 500 µM EDTA.
- For complete transition to the StemFlex™ Medium system from other culture systems, a minimum of two passage adaptation is recommended.

Guidelines for Geltrex™ matrix use

The optimal working concentration of Geltrex™ matrix is cell line dependent. We recommend using a final 1:100 dilution of Geltrex™ matrix in cold DMEM/F-12, GlutaMAX™ Supplement. Coat culture vessels at 37°C, 5% CO_2 for > 1 hour.

Note: Thaw Geltrex™ matrix overnight at 4°C and mix by gentle inversion. Do not allow Geltrex™ matrix to be exposed to room temperature, rather transfer in an ice bucket to the cell culture hood to minimize gelling. Geltrex™ matrix can be combined 1:1 with DMEM/F-12, GlutaMAX™ Supplement, divided into usage size aliquots and stored at –20°C until further use.

Guidelines for rhLaminin-521 use

The optimal working concentration of rhLaminin-521 is cell line dependent.

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 (Cat. No. 14040), DMEM/F-12, GlutaMAX™ Supplement, or StemFlex™ Basal Medium. Do not use complete StemFlex™ Medium. Coat culture vessels at 37°C, 5% CO_2 for > 2 hours

Note: Thaw rhLaminin-521 at 2°C 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.

Recover frozen PSCs in complete StemFlex™ Medium

If using pre-coated plates stored at 2°C to 8°C, pre-warm Geltrex™ matrix plates to room temperature. Pre-warm complete StemFlex™ Medium to room temperature.

1. Pre-coat plates with Geltrex™ matrix. See table for recommended volumes.

   The optimal working concentration of Geltrex™ matrix is cell line dependent.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Geltrex™ matrix[1]	1.5 mL/well	0.6 mL/well	0.3 mL/well	1.5 mL/dish	3 mL/dish	9 mL/dish

[1] Refer to "Guidelines for Geltrex™ matrix use" on page 2 for procedural guidelines.

2. Remove a 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 or 50-mL conical tube and add 3 mL of complete StemFlex™ Medium drop-wise to the cells to reduce 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 StemFlex™ Medium and add to the conical tube containing the 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 StemFlex™ Medium by gently pipetting the cells up and down a few times.

7. Immediately prior to plating of cells and following coating of culture vessel for >1 hour at 37°C, 5% CO_2, aspirate Geltrex™ matrix from the wells and discard. Be certain to not allow the culture surface to dry out.

8. Slowly add the PSC suspension into the Geltrex™ matrix-coated plate, plating ~100,000 viable cells per cm^2 of plate for conditions seeded in the absence of ROCK inhibitor. See table for recommended volumes.

   Note: This amount may need to be adjusted based upon the solution used for cryopreservation.

   (*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 to four days post-thaw. **Do not include additional ROCK inhibitors such as Y-27632 or thiazovivin when using RevitaCell™ Supplement**.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Complete StemFlex™ Medium	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

9. 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.

10. 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 (i.e., 4 mL added per well of 6-well plate). Refer to Figure 1 for flexible feeding schedule.

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

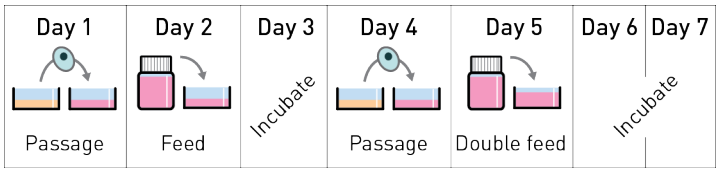


Figure 1 Typical weekly PSC culture workflow using StemFlex™ Medium

Clump cell passage PSCs using Versene Solution or 500 µM EDTA for routine culture

If using pre-coated plates stored at 2°C to 8°C, pre-warm Geltrex™-coated plates to room temperature. Pre-warm StemFlex™ Medium and Versene Solution or 500 µM EDTA solution to room temperature.

1. Pre-coat plates with Geltrex™ matrix. See table for recommended coating conditions.

   The optimal working concentration of Geltrex™ matrix is cell line dependent.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Geltrex™ matrix[1]	1.5 mL/well	0.6 mL/well	0.3 mL/well	1.5 mL/dish	3 mL/dish	9 mL/dish

[1] Refer to "Guidelines for Geltrex™ matrix use" on page 2 for procedural guidelines.

2. Aspirate the spent medium from the vessel containing PSCs and rinse the vessel once with DPBS, no calcium, no magnesium. See table for recommended volumes.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
DPBS(–/–) wash	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

3. Add Versene Solution or 500 µM EDTA to the side of the vessel containing PSCs (refer to table), then swirl the vessel to coat the entire well surface.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Versene Solution or 500 µM EDTA	1 mL/well	0.4 mL/well	0.2 mL/well	1 mL/dish	2 mL/dish	6 mL/dish

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. Remove the cells from the well(s) by gently flushing medium over the surface of the well a few times.

   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 or 500 µM EDTA, and add pre-warmed complete StemFlex™ Medium to the vessel. See table for recommended volumes.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Complete StemFlex™ Medium	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

6. Remove the cells from the well(s) by gently flushing medium over the surface of the well a few times.

7. Collect cells in a 15-mL or 50-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 StemFlex™ Medium to the well(s), which quickly neutralizes the initial effect of the Versene Solution or 500 µM EDTA. 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.

8. Following coating of culture vessel for >1 hour at 37 °C, 5% CO_2, aspirate Geltrex™ matrix from the culture vessel and discard.

   Be certain to not allow the culture surface to dry out.

9. Immediately add an appropriate volume of pre-warmed complete StemFlex™ Medium to each well of a Geltrex™ matrix-coated plate so that each well contains the recommended volume of complete medium after the cell suspension has been added. See table for recommended volumes.

   Note: Step 5 can be completed immediately prior to passaging the cells and cells directly passaged onto the new plate rather than transferring to a conical tube.

   Note: 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.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Complete StemFlex™ Medium	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

10. Move the vessel in several quick side-to-side motions to disperse the cells across the surface of the vessels.

11. Place the vessel gently into the 37°C, 5% CO_2 incubator and incubate the cells overnight.

12. 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 (i.e., 4 mL added per well of 6-well plate).

    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.

Adaptation of mTeSR1 Cultured PSC to StemFlex™ Medium on Geltrex™ matrix-coated vessels

When mTeSR1 cultures achieve passaging confluency (i.e. 60–85% confluency), the cells are ready for adaptation to the StemFlex™ Medium condition.

Passage PSCs as described in "Clump cell passage PSCs using Versene Solution or 500 µM EDTA for routine culture" on page 3.

Note: For complete transition to the StemFlex™ Medium system from mTeSR1 a minimum of two passage adaptation is recommended.

Adaptation of feeder-dependent PSCs to StemFlex™ Medium on Geltrex™ matrix-coated vessels

When the feeder-dependent cultures reach passaging confluency (i.e., 60–85% confluent with round colonies that are not overcrowded), the cells are ready for adaptation to feeder-free culture conditions.

Note: The following instructions are for use of Geltrex™ matrix in transition. For difficult to transition lines, rhLaminin-521 can be implemented for one passage followed by Versene Solution passaging onto Geltrex™ matrix-coated plates.

If using pre-coated plates stored at 2°C to 8°C, pre-warm Geltrex™ matrix-coated plates to room temperature.

1. Pre-coat plates with Geltrex™ matrix. See table for recommended coating conditions.

   The optimal working concentration of Geltrex™ matrix is cell line dependent. See "Guidelines for Geltrex™ matrix use" on page 2

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Geltrex™ matrix	1.5 mL/well	0.6 mL/well	0.3 mL/well	1.5 mL/dish	3 mL/dish	9 mL/dish

   Note: For difficult to transition cell lines, rhLaminin-521 can be implemented for one passage to aid in transition.

2. Prepare a fresh 1 mg/mL Collagenase, Type IV solution in DMEM/F-12, GlutaMAX™ Supplement, filter sterilize using a 0.2-µm filter unit and pre-warm in a 37°C waterbath.

3. Perform manual clean-up of feeder-dependent cultures to remove areas of aberrant differentiation ahead of passaging of cultures.

4. Aspirate the spent medium from the vessel containing PSCs and rinse the vessel once with DPBS, no calcium, no magnesium.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
DPBS (–/–) wash	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

5. Aspirate DPBS, no calcium, no magnesium and add pre-warmed 1 mg/mL Collagenase, Type IV solution.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Pre-warmed Collagenase, Type IV solution	1 mL/well	0.5 mL/well	0.25 mL/well	1 mL/dish	2 mL/dish	6 mL/dish

6. Incubate the dish for ~45 minutes in 37°C, 5% CO_2 incubator.

   Note: Stop the incubation when the edges of the colonies begin to curl from the plate. Do not over-incubate.

7. Add complete StemFlex™ Medium and gently dislodge the colonies from the plate by washing off colonies with a 5-mL serological pipette or 1 mL pipettor.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Complete StemFlex™ Medium	1 mL/well	0.5 mL/well	0.25 mL/well	1 mL/dish	2 mL/dish	6 mL/dish

8. Transfer the suspended colony clusters into a 15-mL conical tube.

9. Add complete StemFlex™ Medium to the vessel to dislodge the remaining colonies and transfer them to the 15-mL conical tube. Repeat trituration of contents transferred to 15-mL conical tube until the desired cluster size is achieved.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Complete StemFlex™ Medium	1 mL/well	0.5 mL/well	0.25 mL/well	1 mL/dish	2 mL/dish	6 mL/dish

10. Let the colony fragments sediment to the bottom of the 15-mL conical tube for 5 minutes by gravity.

11. Discard the supernatant, add complete StemFlex™ Medium, and gently resuspend the sedimented colony fragments by pipetting up and down 2 times.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Complete StemFlex™ Medium	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

12. Gravity sediment the clusters for 2–5 minutes.

13. While the colony fragments are sedimenting, aspirate the matrix solution from the Geltrex™matrix-coated vessel and add complete StemFlex™ Medium.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Complete StemFlex™ Medium	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

14. Aspirate the supernatant and resuspend the sedimented PSC clusters by gently pipetting them up and down 2 times in complete StemFlex™ Medium, taking care not to break them down further.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Complete StemFlex™ Medium	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

15. Distribute resuspended PSC clusters into the Geltrex™ matrix pre-coated vessel, then move the vessel in several quick side-to-side motions to disperse the cells across the surface of the vessel.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Cell suspension	0.5 mL/well	0.25 mL/well	0.125 mL/well	0.5 mL/dish	1 mL/dish	3 mL/dish

16. Place the vessel gently into a 37 °C, 5% CO_2 incubator and incubate the cells overnight.

17. For the first passage post-transition, feed cells daily.

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

18. Passage cells per instructions for "Clump cell passage PSCs using Versene Solution or 500 µM EDTA for routine culture" on page 3 upon attaining ~85% confluency to maintain optimum cell health of cultures.

    Following passaging with Versene Solution, cells can be fed using the optional flexible feed schedules.

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

If using pre-coated plates stored at 2 to 8°C, pre-warm rhLaminin-521-coated plates to room temperature. Pre-warm StemFlex™ Medium and TrypLE™ Select solution to room temperature.

1. Pre-coat plates with rhLaminin-521, refer to recommended coating conditions. See table for recommended volumes.

   The optimal working concentration of rhLaminin-521 is cell line dependent.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
rhLaminin-521[1]	2 mL/well	0.8 mL/well	0.4 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

[1] Refer to "Guidelines for rhLaminin-521 use" on page 2 for procedural guidelines.

2. Aspirate spent medium from the culture vessel.

3. Rinse the vessel once with recommended volume of DPBS, no calcium, no magnesium. See table for recommended volumes.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
DPBS (–/–)	2 mL/well	1 mL/well	0.5 mL/well	2 mL/dish	4 mL/dish	12 mL/dish

4. Add recommended volume of pre-warmed TrypLE™ Select. See table for recommended volumes.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
TrypLE™ Select	1 mL/well	0.4 mL/well	0.2 mL/well	1 mL/dish	2 mL/dish	6 mL/dish

5. Incubate the vessel at 37°C, 5% CO_2 for 5 minutes.

   Note: Note: Avoid extended incubation of PSCs with dissociation reagent to minimize cellular toxicity. Extended incubation time is not necessary.

6. Gently pipette the cells up and down 5–10 times with a 1000 µL pipette to generate a single cell suspension.

7. Transfer the cell suspension to a conical tube containing the recommended neutralization volume of StemFlex™ Medium to dilute the dissociation reagent. See table for recommended volumes.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Neutralization volume, StemFlex™ Medium	3 mL	1.2 mL	0.6 mL	3 mL	6 mL	18 mL

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 resuspension volume of StemFlex™ Medium. See table for recommended volumes.

Culture vessel (surface area)	6-well (10 cm^2)	12-well (4 cm^2)	24-well (2 cm^2)	35-mm (10 cm^2)	60-mm (20 cm^2)	100-mm (60 cm^2)
Resuspension volume, StemFlex™ Medium	2 mL	1 mL	0.5 mL	2 mL	4 mL	12 mL

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 StemFlex™ Medium to achieve the cell seeding density recommended for your culture vessel. See table for seeding densities.

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

[1] 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.

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

12. Immediately prior to plating of cells and following coating of culture vessel for >2 hours at 37 °C, 5% CO_2, aspirate rhLaminin-521 from the wells and discard.

    Do not allow the culture surface to dry out.

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

    Note: If using alternative substrates, such as 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

14. 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.

15. 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 (i.e., 4 mL added per well of 6-well plate).

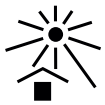
    Note: It is normal to see cell debris and small colonies after passage.

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

Related products

Product	Catalog No.
Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	A14133
DMEM/F-12, GlutaMAX™ Supplement	10565
Recombinant Human Laminin-521	A29248, A29249
DPBS, calcium, magnesium	14040
Versene Solution	15040
DPBS, no calcium, no magnesium	14190
TrypLE™ Select Enzyme (1X), no phenol red	12563
RevitaCell™ Supplement	A26445
PSC Cryopreservation Kit	A26446
Collagenase, Type IV, powder	17104019

Explanation of symbols

				
Temperature Limitation	Manufacturer	Batch Code	Use By	Catalog Number
				
Caution, consult accompanying documents	Consult instructions for use	Keep away from light	Sterilized using aseptic processing techniques	Read Safety Data Sheet

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288