3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Stemline® Pluripotent Stem Cell Culture Medium

Catalog Number **S1202**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Defined, consistent, and feeder independent, Stemline® Pluripotent Stem Cell Culture Medium provides an optimal culture environment for pluripotent cells. Cells cultured in Stemline Pluripotent Stem Cell Culture Medium retain their pluripotent phenotype with minimal spontaneous differentiation, expand at optimal rates, and adapt easily from alternative culture media.

Components

Stemline Pluripotent Stem Cell Culture Medium (Catalog Number S1202) is provided as two components, which are combined for use.

Component	Catalog Number	S1202-100ML	S1202-500ML
Stemline Pluripotent Basal Medium	S0952	100 mL	500 mL
Stemline Pluripotent Supplement, 500×	S1077	0.2 mL	1 mL

Reagents and Equipment Required but Not Provided.

- Y-27632 dihydrochloride (Rho Kinase Inhibitor, Catalog Number Y0503) (Required)
- Accutase® solution (Catalog Number A6964)
- Dimethyl sulfoxide (DMSO, Catalog Number D2650)
- Hanks' Balanced Salt solution (HBSS, Catalog Number H6648)
- Cell culture vessels (plates or flasks) of choice
- ECM Gel from Engelbreth-Holm-Swarm murine sarcoma (Catalog Number E1270)
- CryoStor® cell cryopreservation medium (Catalog Number C2874)
- Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham (DMEM/F12, Catalog Number D6421)
- Corning® cell lifter (Catalog Number CLS3008)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

- Stemline Complete Medium –
  Stemline Pluripotent Stem Cell Culture Medium is supplied as two components, which are combined prior to use.

  1. Thaw the basal medium (Catalog Number S0952) overnight at 2–8 °C.
  2. Thaw the supplement (Catalog Number S1077) at room temperature before addition to the basal medium. Centrifuge the Supplement tube.
  3. Under sterile conditions, transfer the contents of the supplement tube into the bottle of basal medium.
  4. Gently swirl the bottle to mix.

  Note: **Do not refreeze the Stemline Complete Medium.** The Stemline Complete Medium should be stored at 2–8 °C and used within 7 days.

- 10 mM Y-27632 Stock Solution (1000×) –
  Dissolve Y-27632 dihydrochloride (Catalog Number Y0503) in DMSO (1 mg/0.3 mL) and vortex thoroughly.

- Accutase Solution with 10 μM Y-27632 –
  1. Thaw Accutase solution (Catalog Number A6964) overnight at 2–8 °C.
  2. Add 1 μL of 10 mM Y-27632 Stock Solution (1000×) per 1 mL of Accutase solution (final Y-27632 concentration is 10 μM).
  3. Store working aliquots at –20 °C.

- Coating culture vessels with ECM Gel –
  ECM Gel (Catalog Number E1270) is recommended for use with Stemline Complete Medium.
  Note: The ECM Gel will gel within 5 minutes at 20 °C. For prolonged manipulations, work should be conducted below 10 °C under strict aseptic conditions.

  1. Thaw the ECM Gel overnight on ice or at 2–8 °C prior to use.
  2. Dilute the ECM Gel at a 1:100 ratio in ice-cold DMEM/F12 (Catalog Number D6421) and mix well.
  3. Coat each cell culture dish with an appropriate volume of diluted ECM gel to cover the entire surface.
  4. Swirl the culture dish to ensure the entire area is coated sufficiently.
  5. Incubate at 37 °C for one hour prior to use.

Storage/Stability
Stemline Pluripotent Stem Cell Culture Medium components (Catalog Numbers S0952 and S1077) should be stored at –20 °C and protected from light.

After combining the two components, the Stemline Complete Medium can be stored at 2–8 °C and should be used within 7 days.
Note: **Do not refreeze the Stemline Complete Medium.**

Procedure
Please take time to carefully read through these procedures to ensure optimal performance. Stemline Pluripotent Stem Cell Culture Medium provides robust, consistent culturing of human pluripotent stem cells (hPSCs). Contact Sigma-Aldrich Technical support for additional guidance: techserv@sial.com

A. Adaptation of hPSC culture into Stemline Complete Medium
   Stemline Pluripotent Stem Cell Culture Medium allows for stable, confluent culture of hPSCs. Cultures can be transferred from feeder culture or directly from feeder free systems. This can be accomplished by thawing into Stemline Complete Medium or by switching growing cell cultures to Stemline Complete Medium.

1. **Thawing** Cells into Stemline Complete Medium.
   Note: The Stemline Complete Medium should be supplemented with 10 μM Y-27632 to ensure cell survival at seeding**.**

   a. Prepare Stemline Complete Medium with 10 μM Y-27632 – Add 11 μL of 10 mM Y-27632 Stock Solution (1000×) to 11 mL of Stemline Complete Medium.
   b. Allow culture reagents to come to room temperature prior to use (less than 30 minutes). Shield culture reagents from light.
   c. Thaw the cryovial containing the cells by gentle agitation in a 37 °C water bath.
   d. Remove the vial from the water bath as soon as the contents are thawed and spray with 70% ethanol. All operations from this point forward should be carried out under aseptic conditions.
   e. Using a 1 mL pipette, transfer the entire contents of the vial into a 15 mL conical tube.
   f. Slowly add 9 mL of Stemline Complete Medium with 10 μM Y-27632 to the 15 mL conical tube.
   g. Rinse the vial with 1 mL of Stemline Complete Medium with 10 μM Y-27632 and add this to the 15 mL conical tube.
   h. Centrifuge the cells for 5 minutes at 200 × *g*.
   i. Aspirate the medium and resuspend the cells in 1 mL of Stemline Complete Medium with 10 μM Y-27632.
   j. Seed cells to ECM-coated cell culture dishes at a density of ~20,000 cells/cm^2 in Stemline Complete Medium with 10 μM Y-27632.
   k. Change medium daily, see procedure B, Feeding.

2. **Switching cultures** to Stemline Complete Medium with 10 μM Y-27632. Sub-confluent hPSCs can be switched to Stemline Pluripotent Stem Cell Culture Medium at any time.

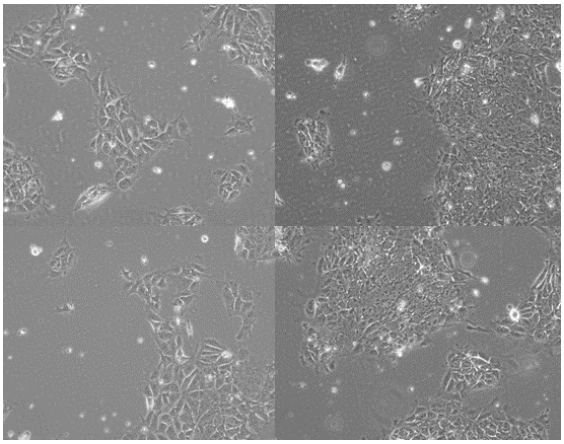
B. Feeding – Stemline Complete Medium **Change Frequency**: Daily replacement of the culture medium is recommended. A volume of ~0.2 mL of Stemline Complete Medium **without** Y-27632 should be used for each square centimeter (cm^2) of culture surface.

C. Passaging

Notes: Human iPS cells typically need to be passaged every 3–4 days. If the culture is allowed to overgrow, the iPS cells will begin to differentiate. The cells should be passaged when the culture reaches ~80% confluence. hPSCs in Stemline Complete Medium may be passaged by enzymatic (using Accutase) or mechanical dissociation.

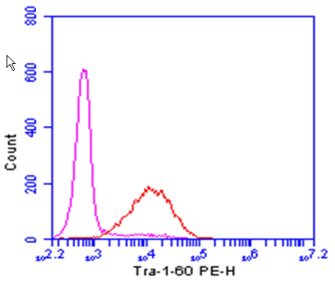
At low density, a flattened, spread out cell morphology is characteristic of hPSCs cultured in Stemline Complete Medium, while at higher density the cells are more closely packed. (see Figure 1).

Figure 1.
PSC morphology in Stemline Pluripotent Stem Cell Culture Medium



Human iPS cells (Catalog Number IPSC0028) were seeded at 20,000 cells/cm^2. Images (10×) were taken at day 1 and 2 of culture.

Figure 2.
PSC express pluripotency marker Tra-1-60 when cultured in Stemline Pluripotent Stem Cell Culture Medium.



Human iPSC (Catalog NumberIPSC0028) cultured in Stemline Pluripotent Stem Cell Culture Medium for 5 passages were analyzed by flow cytometry to detect the percentage of cell population expressing the pluripotency marker Tra-1-60. The histogram shows over 90% of the cells express Tra-1-60 (red) when compared to the unstained (isotype control – pink) cells.

1. Enzymatic Dissociation
   Note: For enzymatic dissociation of hPSCs, use the Accutase Solution with 10 μM Y-27632 and Stemline Complete Medium with 10 μM Y-27632 to ensure cell survival.

   a. Under aseptic conditions, remove culture medium from cells, rinse with pre-warmed HBSS (Catalog Number H6648), and aspirate buffer.
   b. Dissociate cells using pre-warmed Accutase Solution with 10 μM Y-27632 for 3–5 minutes at 37 °C.
   c. Add an equal volume of Stemline Complete Medium with 10 μM Y-27632.
   d. If the cells are not fully detached from the cell culture surface, gently lift them off using a cell lifter (Catalog Number CLS3008).
   e. Transfer the entire contents to a 15 mL conical tube and centrifuge for 5 minutes at 200 × *g*.
   f. Aspirate the medium and resuspend the cells in 1 mL of Stemline Complete Medium with 10 μM Y-27632.
   g. Seed cells to an ECM-coated cell culture dish at a density of ~20,000 cells/cm^2 with Stemline Complete Medium with 10 μM Y-27632.
   h. Change medium daily, see procedure B, Feeding.

2. Mechanical Dissociation
   a. Under aseptic conditions, remove culture medium from the cells aseptically, rinse with pre-warmed HBSS, and aspirate buffer.
   b. Add fresh Stemline Complete Medium with 10 μM Y-27632.
   c. Using a sterile pipette tip or a flame drawn glass pipette, gently score the colonies to break into clusters of a few hundred cells.
   d. Gently lift cells off using a cell lifter (Catalog Number CLS3008).
   e. Gently pipette the cells up and down several times to mix thoroughly and break up larger clumps.
   f. Seed cells to an ECM-coated cell culture dish at the desired cell density in Stemline Complete Medium with 10 μM Y-27632.
   g. Change medium daily, see procedure B, Feeding.

D. Cell cryopreservation
   After enzymatic or mechanical dissociation, cells can be cryopreserved.

   1. Dissociate cells as described in Procedure C, Passaging.
   2. Transfer the cells to a 15 mL conical tube and centrifuge for 5 minutes at 200 × *g*.
   3. Aspirate the medium and resuspend the cells in cold CryoStor cell cryopreservation medium (Catalog Number C2874) at 1×10^6 cells/mL.
   4. Transfer the cells to a cryovial and place in an appropriate freezing container at –80 °C. For longer term storage, it is recommended to store the cells in liquid nitrogen.

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