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Product Information

iPSC β-ACTIN GFP
Induced Pluripotent Stem Cells

Catalog Number **IPSC1030**
Storage Temperature –196 °C, liquid nitrogen

TECHNICAL BULLETIN

Product Description

Induced pluripotent stem cells (iPS cells or iPSCs) are derived from adult somatic cells by induction of expression of specific genes.[1-2] Similar to embryonic stem cells, iPSCs are capable of differentiating into multiple cell lineages representing all three germ layers, and can form embryoid bodies and teratomas.

iPSC β-ACTIN GFP cells are produced by OSKM retrovirus reprogramming of epithelial cells from a Caucasian female 24 years of age with no known genetic disorders. iPSC β-ACTIN GFP cells have *ACTB* regulated expression of GFP. Six different isoforms of actin are known in humans. β-Actin is one of two non-muscle cytoskeletal actins involved in cell motility, structure and integrity.

Cell Line Description
iPS cells
Source Tissue: Epithelium
Gender: Female
Age: 24
Ethnicity: Caucasian
Reprogramming method used: OSKM retrovirus

Component

iPSC β-ACTIN GFP (human iPSCs, >1.0 × 10^6 cells) Catalog Number IPSC1030 Frozen as a suspension of single cells in CryoStor® CS10 cell cryopreservation medium (Catalog Number C2874)	1 vial

Reagents and Equipment Required but Not Provided.

Products for Feeder-Free Stem Cell Culture System
Stemline® Pluripotent Stem Cell Culture Medium (Catalog Number S1202)
ECM Gel from Engelbreth-Holm-Swarm murine sarcoma (Catalog Number E1270)
DMEM/F12 medium (Catalog Number D6421)
Hanks' Balanced Salt solution (HBSS, Catalog Number H6648)
Y-27632 dihydrochloride (Catalog Number Y0503)
Accutase® solution (Catalog Number A6964)
CryoStor® CS10 cell cryopreservation medium (Catalog Number C2874)
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.

Biosafety Level: 1
This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Appropriate safety procedures are recommended to be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures have been published.[3-6]

Preparation Instructions

10 mM Y-27632 Stock Solution (1,000×) – Dissolve Y-27632 dihydrochloride (Catalog Number Y0503) in DMSO (1 mg/0.3 mL) and vortex thoroughly.

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 14 days.

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 (1,000×) 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, it should be maintained 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 100-fold with ice-cold DMEM/F12 medium (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.
6. Aspirate the ECM and rinse with phosphate buffered saline.
7. Add warm Stemline Complete Medium with 10 μM Y-27632 to the dish.

Unused ECM coated plates may be stored at 37 °C in serum free medium for up to 7 days.

Storage/Stability

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase. Extended storage at –70 °C will result in loss of viability.

Procedures

Please take time to carefully read through these procedures to ensure optimal performance. Human iPSC β-ACTIN GFP cells expand optimally in Stemline Pluripotent Stem Cell Culture Medium. Contact Sigma-Aldrich Technical support for additional guidance: techserv@sial.com

Thawing iPSC β-ACTIN GFP Cells into Stemline Complete Medium

Note: Thaw into Stemline Complete Medium supplemented with 10 μM Y-27632 to improve cell survival.

1. Prepare Stemline Complete Medium with 10 μM Y-27632 – Add 11 μL of 10 mM Y-27632 Stock Solution (1,000×) to 11 mL of Stemline Complete Medium.
2. Allow culture reagents to come to room temperature prior to use (less than 30 minutes). Shield culture reagents from light.
3. Thaw the cryovial containing the iPSC β-ACTIN GFP cells by gentle agitation in a 37 °C water bath.
4. 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 aseptically.
5. Using a 1 mL pipette, transfer the entire contents of the vial into a 15 mL conical tube.
6. Slowly add 9 mL of Stemline Complete Medium with 10 μM Y-27632 to the 15 mL conical tube.
7. Rinse the vial with 1 mL of Stemline Complete Medium with 10 μM Y-27632 and add this to the 15 mL conical tube.
8. Centrifuge the cells for 5 minutes at 200 × *g*.
9. Aspirate the medium and resuspend the cells in 1 mL of Stemline Complete Medium with 10 μM Y-27632.
10. 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. Incubate at 37 °C in a 5% CO_2 atmosphere.
11. Change medium daily, see Procedure, Feeding.

Feeding iPSC β-ACTIN GFP Cells – 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.

Passaging

Note: Human iPSC β-ACTIN GFP 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. When cultured with Stemline Pluripotent Stem Cell Medium, iPSC β-ACTIN GFP cells may be passaged by enzymatic (using Accutase) or mechanical dissociation.

1. Enzymatic Dissociation
   Note: For enzymatic dissociation of iPSC β-ACTIN GFP cells, use the Accutase Solution with 10 μM Y-27632 and Stemline Pluripotent Stem Cell Culture 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 Pluripotent Stem Cell Culture 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 Pluripotent Stem Cell Culture 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 Pluripotent Stem Cell Culture Medium with 10 μM Y-27632.
   h. Change medium daily, see Procedure, 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 Pluripotent Stem Cell Culture 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, Feeding.

Cell cryopreservation

After enzymatic or mechanical dissociation, cells can be cryopreserved.

1. Dissociate cells as described in Procedure, 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) with 10 μM Y-27632 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.

References

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3. Fleming, D.O. et al., (1995) Laboratory Safety: Principles and Practice. 2nd edition, ASM press, Washington, DC.
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5. Caputo, J.L., Biosafety procedures in cell culture. J. Tissue Culture Methods, **11**, 223-227 (1998).
6. Centers for Disease Control (1999), Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 93-8395. U.S. Dept of Health and Human Services; 4th Edition U.S. Government Printing Office, Washington, D.C. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

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