H9 Cell Line

Cat. No. : CL-0890

Origin and General Characteristics

Cell Name	H9
Organism	Human
Age	Blastocyst
Sex	Female
Tissue	Embryo
Morphology	Spherical colonies
Growth Properties	Adherent
Background	The H9 human embryonic stem cell line is one of the most widely used human embryonic stem cell lines worldwide. This cell line was derived from the inner cell mass of a human blastocyst and exhibits unlimited proliferation, self-renewal, and multilineage differentiation in vitro, providing reliable experimental material for stem cell research.
Biosafety Level	BSL-1

Culture Conditions and Handling

Complete Medium	Complete culture medium for H9[CM-0890]
Subcultivation Ratio	1:2-1:4
Medium Renewal	Once daily
Dissociation Duration	3-5 min
Incubation Atmosphere	Air, 95%; CO_2, 5%
Temperature	37°C
Freeze medium	90% embryonic complete stem cell medium + 10% DMSO + Y-27632 dihydrochloride solution[PB180609]
Storage Conditions	For long-term cryopreservation, cryovials should be stored in liquid nitrogen at −150°C to −196°C. Storage at −80°C is restricted to short-term interim use only.
Instructions	1. Check all containers for leakage or breakage. 2. Remove the frozen cells from the dry ice packaging and immediately transfer them to liquid nitrogen (liquid or vapor phase) for long-term cryopreservation. 3. Abundant debris appears during culture of this cell line; timely medium exchange and washing are required. Perform cell recovery and culture only after all preparations are complete. 4. The working concentration of Y-27632 dihydrochloride solution is 10 μM. In stem cell culture, Y-27632 dihydrochloride solution is used during cryopreservation, recovery, and subculture; it is not required during medium exchange. 5. For culturing this cell line, please prepare the following in advance: Stem Cells Planking Solution (1 ×) [Cat. No.:PB180537], Y-27632 Dihydrochloride Solution [Cat. No.: PB180609], and stem cell dissociation solutions. Options include: Accutase Cell Dissociation Solution [Cat. No.: PB180201], Recombinant Trypsin

Solution, with phenol red[Cat. No.:PB180241], and Recombinant Trypsin Solution, without phenol red[Cat. No.:PB180240].

Handling Recommendations for Cryopreserved Cells

Preparations Before Thawing

1. Transfer 50 mL complete stem cell medium into a 50 mL centrifuge tube, add 50 µL Y-27632 dihydrochloride solution (10 mM) [Cat. No.: PB180609], mix thoroughly and prewarm for standby use.
2. Coat one well of a 6-well plate with stem cells planking solution (1×)[Cat. No.:PB180537]. Add 1 mL planking solution to the well and rock the plate to evenly cover the bottom surface. Incubate at 37°C for 2 h before use. (If the coated well is not for immediate use, seal tightly to prevent dehydration. Coated plates remain stored at 2 – 8°C for up to 7 days. Before using the stored plates, equilibrate at 37°C for 30 min in advance, then aspirate excess matrix gel. Avoid scratching the coated surface with pipette tips.)

Thawing Procedure

1. Retrieve the cryovial containing frozen cells from liquid nitrogen storage. Place it in sterile gloves and immediately submerge it in a 37°C water bath. Thaw the cells rapidly (<1 minute) by gently swirling the vial in the 37°C water bath until only a small ice crystal remains. Transfer the vial to a biosafety cabinet. Wipe the exterior of the vial with 75% ethanol before opening.Transfer the entire thawed cell suspension to a 15 mL centrifuge tube using a single-channel pipette.
2. Inside the biosafety cabinet, slowly add 5 mL complete stem cell medium supplemented with Y-27632 dihydrochloride solution (10 mM) [Cat. No.: PB180609] dropwise to the 15 mL tube containing the cell suspension (corresponding to a 500 µL cryovial volume). Cap the tube and invert gently 3 times to mix. Centrifuge at 300 × g for 3 min at room temperature to pellet cells.
3. Inside the biosafety cabinet, carefully aspirate the supernatant. Resuspend the cell pellet in 1 mL fresh complete stem cell medium containing Y-27632 dihydrochloride solution (10 mM) [Cat. No.: PB180609], then transfer the suspension into one well of a pre-coated 6-well plate pre-filled with an additional 1 mL of the same supplemented medium. Label the plate with cell line name, thaw date, and passage number, and incubate at 37°C with 5% CO_2 and saturated humidity.

Medium Exchange

1. Cells start attaching within 30 min post-recovery and complete adherence after 8 h. Daily medium replacement is mandatory due to heavy debris accumulation.
2. Inside the biosafety cabinet, aspirate and discard spent medium. Rinse the cell monolayer with DPBS and remove the wash buffer. Add 1 mL fresh Y-27632-free complete medium to the well. Note: Y-27632 dihydrochloride solution is not added to medium for routine medium exchange.

Subculturing Procedure

1. Perform subculture when cell confluency reaches 70%–80%. Aspirate all spent medium from the well of the 6-well plate, and rinse the cell monolayer 1–2 times with 1 mL 1× PBS to remove residual medium.
2. Add 0.5 mL stem cell dissociation solution, rock the plate gently to fully cover cells, and incubate at 37°C for 3–5 min. Discard dissociation solution once obvious cell retraction is observed under a microscope. Gently triturate cells with fresh medium to prepare cell suspension (single-cell dissociation is not required). Split ratio guidance: Use 1:4 for the first subculture. Increase the ratio

if cells reach full confluency within 2 days; reduce the ratio if cells remain subconfluent after 3–4 days.

Cell Cryopreservation

1. Cryopreserve cells at 70% – 80% confluency (per single well of a 6-well plate); avoid full 100% confluency.
2. Inside the biosafety cabinet, aspirate and discard spent medium, rinse cells 1–2 times with 1 mL 1 × PBS, then add 0.5 mL stem cell dissociation solution and incubate for 3–5 min. Triturate to form a cell suspension, neutralize digestion with complete medium, and transfer all liquid to a 15 mL or 50 mL centrifuge tube.
3. Centrifuge the cell suspension at 300 × g for 3 min at room temperature. Open the tube and aspirate the supernatant, then resuspend the pellet in freezing medium supplemented with Y-27632 dihydrochloride solution [Cat. No.: PB180609]. Aliquot the suspension into two cryovials (standard yield: 2 cryovials per single well of a 6-well plate).
4. Place cryovials into a controlled-rate freezing container, complete the cell cryopreservation log, and store at –80°C overnight. Transfer vials to liquid nitrogen storage the following day.

Precautions After Receipt of Frozen Cells

1. Upon unpacking, inspect the condition of the frozen cells and dry ice, and take photographs immediately. The following after-sales service will be provided based on these photographs, including assessment of the remaining dry ice, verification that the cryovial was fully buried in dry ice, and evaluation of whether the cells thawed and refrozen during transit.
2. Upon receipt, the cells should be transferred to liquid nitrogen immediately or directly resuscitated. If liquid nitrogen is unavailable, the cells can be temporarily stored at -80°C, however, the storage period should be limited to less than two weeks whenever possible. Prolonged storage at -80°C may gradually reduce post-thaw cell viability, and the extent of this viability loss is unpredictable.
3. Ensure that the operator has sufficient knowledge and experience in cell culture, and the laboratory is equipped with essential instruments, including a biosafety cabinet, CO_2 incubator, inverted microscope, centrifuge, water bath. Carefully review the cell instruction manual to understand key cell characteristics including growth properties (adherent or suspension), morphology, basal medium requirements, serum concentration, cytokine supplementation, subcultivation ratio, medium renewal schedule.
4. After resuscitation, observe the cells under a microscope and record the cell status by taking photographs (1-3 images each at 100× and 200× magnification for 3 consecutive days). These images will serve as supporting documentation for follow-up services. In addition, a small aliquot of cells may be used to assess cell viability by automated cell counting or trypan blue staining.
   Note: Cells should not be observed too frequently within the first 24 hours after resuscitation, as this may affect cell growth or adherence. Observation once per day is sufficient.
5. After sufficient expansion, the cells should be cryopreserved as soon as possible to ensure availability of backup stocks for future experiments or in case of unexpected experimental failure. Cells in the logarithmic growth phase should be selected for cryopreservation. After cryopreservation, it is recommended to resuscitate one vial to verify post-thaw viability and confirm successful cryopreservation.