EZ-Stem™ Complete Medium Product Instruction

Product Information

Catalog	YM-HA-001		
Product	EZ-Stem™ Complete Medium		
Components 1	Basal Medium For ESC and IPSC	Quantity	400 mL
Components 2	Supplement C	Quantity	20 mL
Components 3	Supplement D	Quantity	80 mL
Catalog	YMH-002		
Product	EZ-Stem™ Cryopreservation Medium		
Components	EZ-Stem™ Cryopreservation Medium	Quantity	50 mL
Catalog	YMH-003		
Product	EZ-Stem™ Passaging Medium		
Components	EZ-Stem™ Passaging Medium	Quantity	500 mL
Catalog	YMH-004		
Product	EZ-Stem™ Apoptosis inhibitor Medium		
Components	EZ-Stem™ Apoptosis inhibitor Medium	Quantity	1 mL
Quality Control	Passed bacterial, fungal, mycoplasma, and endotoxin tests. Passed osmotic pressure and pH testing. Passed product performance testing.		

Preservation

1. All the Components in the set should avoid direct exposure to sunlight.

Basal Medium For Human ESC and IPSC in the set should be stored at 4℃, shelf life: 1 year； Supplement C and Supplement D should be stored at -20℃, shelf life: 1 year；

2. Thaw the Supplement C/D at 4℃ condition, not 37℃. (Note: Supplement C/D should not undergo the thawing process more than 2 times.)
3. After preparation, the EZ-Stem™ Complete Medium should be stored at 4℃, shelf life: 2 weeks;
4. Please use the product within its shelf life. Expired Components may seriously affect the culture effect.

Preparation of EZ-Stem™ Cell Complete Medium

Mix Basal Medium For Human ESC and IPSC, Supplement C and Supplement D to prepare EZ-Stem™ Cell Complete Culture Medium, which can be stored at 2 ℃ to 8 ℃ and shall be used up within 2 weeks.

Preparation

1. Aliquot Matrigel (Catalog: 354277)

Place Matrigel in an ice box one day in advance and then put the ice box into a 4℃ refrigerator. The frozen Matrigel must be thawed into liquid, and the pipette tip and EP tube used for aliquot should be placed at -20℃ in advance. During the aliquot operation, do not touch the Matrigel with your hands to prevent the Matrigel from solidifying due to body temperature; The whole aliquot process must be carried out on ice, and the sufficient aliquots will be used according to the requirement for each experiment. Store the aliquots at -20℃,

thaw in 4°C refrigerator for 15min before use.

2. Coating

1) Thaw the Matrigel in 4°C refrigerator in advance. Precool the DMEM/F12 basal medium and prepare centrifuge tube and 6-well plate.

2) Dilute the Matrigel according to the ratio of Matrigel:DMEM/F12 basal medium =1:100. The detailed operation is as follows (taking the preparation of 50ml/tube as an example): Take 50ml of pre-cooled DMEM/F12 basal medium into the centrifuge tube, use the pipette to take 700ul of pre-cooled DMEM/F12 basal medium from the centrifuge tube to the Matrigel aliquot, mix gently by pipetting until there is no obvious colloid, then add the Matrigel solution to the centrifuge tube with the remaining DMEM/F12 basal medium, and mix it well by upside down for 20 times.

3) Add the diluted, Matrigel-containing medium to the culture plate that requires coating, add 1ml to each well of the 6-well plate, and other sizes of culture plate follow the same ratio. Place the plates in 37°C incubator, and use it after 2 hours.

4) If the coated plate would not be used immediately, add 1ml DMEM/F12 to each well of the 6-well plate. After sealing with a sealing film, it can be stored in a 37°C incubator for one week. If the diluted Matrigel would not be used immediately, it can be stored at 4 degrees and should be used as soon as possible. The longer the storage time, the worse the coating effect.

Cell Thawing (Based on 6-well plate)

1. Preparation: Preheat the EZ-Stem™ Cell Complete Medium at room temperature in advance.Take the cryopreserved cells from liquid nitrogen to a box filled with dry ice,

and leave for several minutes to volatilize residual liquid nitrogen.

2. Take out the cryopreserved cells from dry ice, shake slightly before thawing to remove residual dry ice. Then hold the cap with forceps, quickly thaw cells in a 37°C water bath by gently swirling the vial (Note: keep the cap out of the water). In about 1 minute, it would completely thaw; Inside the ultra-clean bench, sterilize the outer surface of the vial by wiping with an alcohol cotton pellet and leave it to dry. Transfer the thawed cell suspension to a 15ml centrifuge tube.

3. Inside the ultra-clean bench, get 5ml EZ-Stem™ Complete Medium (corresponding to 300ul of cryopreservation solution) and add the medium dropwise by a pipette to the 15ml centrifuge tube which already contains the thawed cell suspension, close the lid, mix by inverting up and down for 3 times, gently and slowly. Then centrifuge at 700rpm for 5 mins at room temp to collect the cells.

4. Inside the ultra-clean bench, carefully remove and discard the supernatant. Resuspend cell pellet with 1ml of EZ-Stem™ Complete Medium (EZ-Stem™ Apoptosis inhibitor Medium-containing) and then transfer to a 6-well plate containing 1 ml of EZ-Stem™ Complete Medium (EZ-Stem™ Apoptosis inhibitor Medium-containing), label the plate with cell name, date and passage no., incubate the plate in a 37℃, 5%CO2 incubator.

Cell Passaging

1. As long as the cells are 70%-80% confluent, it is ready to passage. Inside the ultra-clean bench, remove and discard the medium from the 6-well plate and briefly rinse the cells with 1ml of 1×PBS for 1-2 times to remove residual medium.

2. Add 0.5ml of EZ-Stem™ Passaging Medium, gently shake the plate to allow EZ-Stem™ Passaging Medium completely cover the cells, place the plate into the 37℃ incubator and incubate for 5-10 mins until the majority of the cells have significant retraction (colony pulling away from the matrix layer) under the microscope, remove and discard

the EZ-Stem™ Passaging Medium. Then add the fresh EZ-Stem™ Complete Medium, gently pipet the cells to cell suspension (do not make it into single cell), and passage the cells at appropriate passage ratio.

3. 1:6-1:8 for the first passage, increasing the passaging ratio if the cells are grown to confluence within two days, or decreasing the passaging ratio if the cells are not grown to confluence in 3-4 days.

Cell cryopreservation

1. Based on the 6-well plate, as long as the cells are 70%-80% confluent (not 100%), it is feasible for cryopreservation. Inside the ultra-clean bench, add 1×PBS to the 6-well plate and briefly rinse the cell 1-2 times, add EZ-Stem™ Passaging Medium for digestion for 5-10 mins. When most cells become bright and round, and the cells have not yet detached from the coated surface or floated, the digestion is completed.
2. Upon digestion, carefully aspirate the EZ-Stem™ Passaging Medium
3. Take the EZ-Stem™ Cryopreservation Medium, add 1 ml to each well, mix gently by pipetting, horizontally swirl the plate (up and down, left and right) for 3 times, then transfer the cell suspension to the cryopreservation tubes.
4. Place the cryovials in a cell freezing container, put the container in a -80℃ freezer overnight, then transfer the cryovials to liquid nitrogen on the next day.

Note: EZ-Stem™ Stem Cell Apoptosis Inhibitor is a widely used non-muscle myosin II ATPase inhibitor that acts on the ROCK-myosin II signaling pathway and can prevent apoptosis caused by isolation of pluripotent stem cells. It can improve the survival rate and cloning efficiency of isolated hPSCs without affecting the self-renewal or pluripotent properties of stem cells.

Special Note

1. Ensure that the EZ-Stem™ Cell Complete Medium is stored at 4 ℃ and used up within 2 weeks. Only preheat the medium required for the current experiment each time to reduce temperature changes in the EZ-Stem™ Cell Complete Medium and avoid a decrease in factor potency in the medium.
2. If the entire culture medium cannot be used up in the short term, we suggest preparing it in batches. Please prepare the required amount according to the proportion of each component in the set. However, the remaining Components must be stored strictly according to their respective storage conditions and cannot be frozen or thawed multiple times.
3. All components in the EZ-Stem™ Cell Complete Medium kit are strictly controlled for sterility, and we generally do not recommend further sterilization. If there is a risk of contamination during the preparation process, EZ-Stem™ Cell Complete Medium can be filtered for sterilization.