Cell Use Instruction - H9 Cell Line

Product Info

Catalog	YC-C097		
Cell line	H9		
Morphology	Spherical clone	Passage ratio	1:6-1:8
Culture method	EZ-Stem™ Cell Culture Medium Ubigene didn't use P/S. But client could use P/S after cells grow in good condition after thawing.		
Cryopreservation solution	EZ-Stem™ Cryopreservation Medium		
Special Note			

STR Authentication

Loci	STR Info (Sample Cell) Sample Cell Line: H9			STR Info (Cell bank) Cell Line: WAe009-A-5		
	Allele1	Allele2	Allele3	Allele1	Allele2	Allele3
D5S818	11	12		11	12	
D13S317	9	9		9	9	
D7S820	9	11		9	11	
D16S539	12	13		12	13	
VWA	17	17		17	17	
TH01	9.3	9.3		9.3	9.3	
AMEL	X	X		X	X	
TPOX	10	11		10	11	
CSF1PO	11	11		11	11	

* STR authentication data of this cell line matches with that of cell lines sourced from ATCC, DSMZ, JCRB, and RIKEN databases.

Karyotype analysis

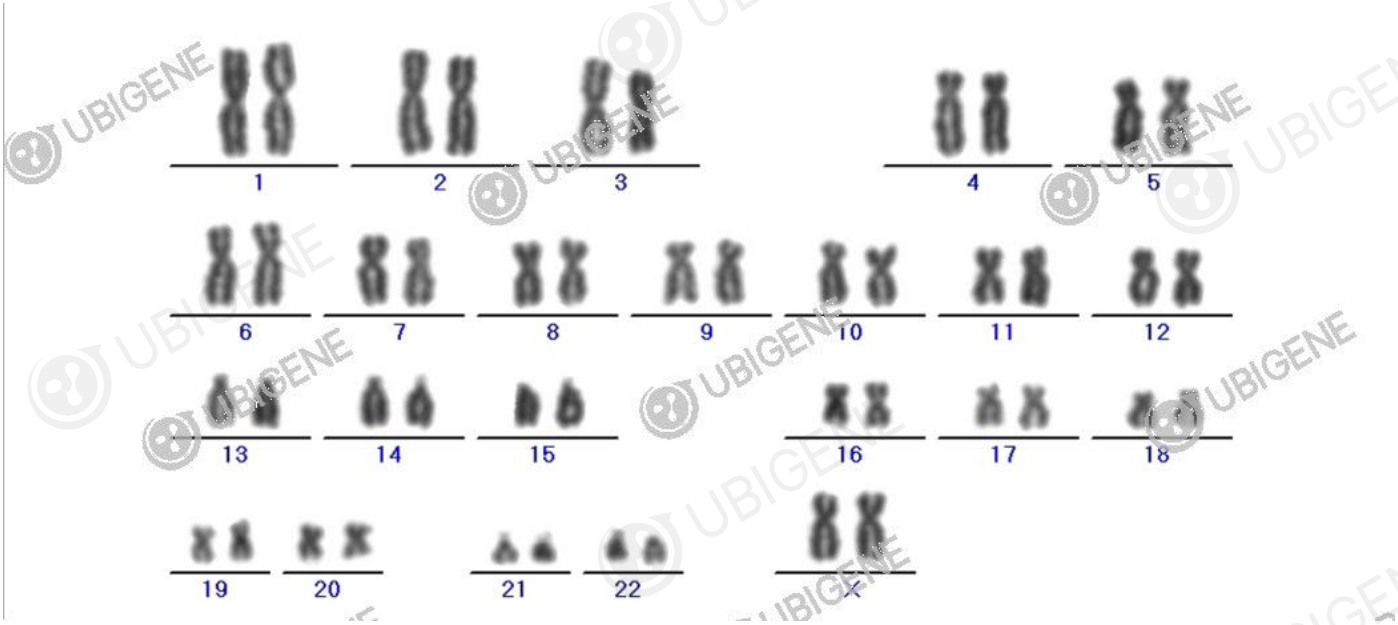

Figure 1 Cell chromosome map 1 (2n=46) of H9

Cell Reception

Cryopreserved cells:

In the case of cryopreserved cells transported with dry ice, upon received, immediately transfer to liquid nitrogen for storage or store briefly at -80°C freezer, or proceed directly to cell thawing. Upon cell thawing, please count the cell number and cell viability and take some photos of the cells under different magnification (e.g. at 100x and 40x) as the records.

Notice: Upon received, please ensure to takes photos of the package, including dry ice and the tubes, and contact us within 24 hrs if any abnormalities such as dry ice has ran out, the cap of the cryovial is dislodged, broken and the cell is contaminated.

Cell Thawing (Based on 6-well plate)

1)Preparation:

Preheat the EZ-Stem Complete Medium at room temperature in advance. Take the cryopreserved vial from liquid nitrogen to a box filled with dry ice, and leave for several minutes to volatilize residual liquid nitrogen;

The 6-well plate needs to be coated with Matrigel, which must be dissolved at 4°C, and the coating process should be completed in a short time, because the Matrigel will solidify soon when temperature above 10°C. Prepare the solution following the ratio of the Matrigel versus DMEM/F12 basal medium=1:100, mix well and add 1ml of Matrigel solution to each well of a 6-well plate after mixing, shake to make the bottom surface be coverred evenly. It can be used after being placed at 37°C for two hours.

2)Take out the cryopreserved vial 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 80%-90% 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 3-5 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, then gently pipet the cells to the single cell suspension and add the fresh EZ-Stem Complete Medium to terminate digestion. All liquid is transferred to a 15ml or 50 ml centrifuge tube.

2)Centrifuge at 300g for 3 mins at room temp. After centrifugation, remove and discard the supernatant, and add 600 μL EZ-Stem Cryopreservation Medium and 0.15 μL EZ-Stem Apoptosis inhibitor Medium then aliquot into 2 vials (each well can cryopreserve 2 vials).

3)Place the cryovials in a cell freezing container, record the information for the cryopreserved cells, put the container in a -80℃ freezer overnight, then transfer the cryovials to liquid nitrogen on the next day.