2018

BIOMEDICUM

Stem Cell Center

HUMAN PLURIPOTENT STEM CELLS

THAWING hiPSC TO MATRIGEL™ DISHES/PLATES

hiPSC are extremely fragile following thawing. Handle them gently. Prepare all reagents in advance so that as soon as the last ice crystal is thawed, you are diluting the DMEM/F12 media/ESSENTIAL 8TM MEDIUM.

Materials and media needed:

- +37^{0}C water bath
- 70 % ethanol
- 15 ml conical centrifuge tube
- Matrigel™ plate(s)/dish(es) (see SOP 'Reagents and media preparation/Matrigel™ basement membrane matrix')
- DMEM/F12 with GlutaMAX media
- ESSENTIAL 8TM MEDIUM (see SOP 'Reagents and media preparation/ESSENTIAL 8™ MEDIUM')
- ROCK inhibitor 10 mM (see SOP 'Reagents and media preparation/ROCK inhibitor Y-27632 2HCl')
- centrifuge
- incubator (+37°C, 5 % CO_2)

Procedure:

1. Rinse Matrigel™ dishes/plates gently once with serum-free DMEM/F12 with GlutaMAX media before adding 1 ml cell culture media and 2 µl ROCK inhibitor.
2. Add 5 ml of DMEM/F12 with GlutaMAX media/ ESSENTIAL 8TM MEDIUM into a 15 ml conical centrifuge tube.
3. Remove cell vial from -150ºC or liquid nitrogen.
4. Thaw vial quickly in +37ºC water bath.
5. When just a small crystal of ice remains, treat the outside of the vial with 70 % ethanol. Wipe ethanol away before opening the vial.
6. Very gently, move cells from the vial into a 15 ml conical centrifuge tube (with pre-added 5 ml of DMEM/F12 with GlutaMAX media/ ESSENTIAL 8TM MEDIUM). While adding cells, gently mix the suspension in the tube by gently tapping the tube with a finger.
7. Centrifuge 500 rpm for 5 min RT.
8. Discard supernatant.
9. Re-suspend cell pellet in 1 ml of ESSENTIAL 8™ MEDIUM culture media and add all cells to a Matrigel™ ø 3,5 cm culture dish.
10. Place cell culture dish into an incubator set at +37ºC with 5 % CO_2 in air.
11. Change the media next day to remove dead cells. From now on

change the media every day/every other day.

PASSAGING hiPSC (EDTA)

Materials and media needed:

- 0,5 mM EDTA (see SOP 'Reagents and media preparation/EDTA')
- Matrigel™ plate(s)/dish(es) (see SOP 'Reagents and media preparation/Matrigel™ basement membrane matrix')
- DMEM/F12 media with GlutaMAX
- ESSENTIAL 8™ MEDIUM (see SOP 'Reagents and media preparation/ ESSENTIAL 8™ MEDIUM hESC')
- incubator (+37ºC, 5 % CO_2)

Procedure:

1. Rinse Matrigel™ dishes/plates gently once with DMEM/F12 with GlutaMAX media before adding 1 ml cell culture media.
2. Rinse cells with 0,5 mM EDTA two times (0,5ml per ø 3,5 cm/1 ml per ø 6cm cell culture dish).
3. Add 0,5 mM EDTA and incubate the cells for approximately 3–5 min in incubator. When the cells start to separate and round up, and the colonies will appear to have holes in them when viewed under a microscope, they are ready to be removed from the dish.
4. Remove EDTA.
5. Add culture medium and gently re-suspend cells by triturating gently with a pipette.
6. Divide cell suspension to e.g. two rinsed Matrigel™ dishes (final dilution of 1:2). Passaging ratio depends on the cell growth and will vary (1:2–1:10).
7. Place cell culture dish into an incubator set at +37ºC with 5 % CO_2 in air.
8. Change the media next day to remove dead cells. From now on change the media every day/every other day.

NOTE: To be sure the colonies are small enough; check them by closing the lid tightly and looking at colonies under the microscope before next step. Colonies that are not broken up are likely to differentiate.

NOTE: If the treatment with 0,5 mM EDTA seems insufficient to remove the cells from the dish use a cell lifter to gently scrape the colonies from the dish instead of breaking the colonies into too small pieces by triturating harshly with a pipette.

FREEZING hPSC

Materials and media needed:

- cryovials
- 0,5 mM EDTA (see SOP ‘Reagents and media preparation/EDTA’)
- (cell lifter/scraper)
- 15 ml conical centrifuge tube
- DMEM-F12 media with GlutaMAX
- centrifuge
- freezing medias I and II (see SOP ‘Reagent and media preparation /Freezing medias for hiPS cells’)
- -80°C
- liquid nitrogen

Procedure:

1. Pre-label cryovials.
2. Rinse cells with 0,5 mM EDTA two times (0,5ml per ø 3,5 cm/1 ml per ø 6 cm cell culture dish).
3. Add 0,5 mM EDTA and incubate the cells for approximately 3–5 min in incubator. When the cells start to separate and round up, and the colonies will appear to have holes in them when viewed under a microscope, they are ready to be removed from the dish.
4. Remove EDTA.
5. Gently re-suspend cells in 0,5 ml Freezing media I per vial (half of the final volume needed for freezing) and remove suspension to a cryovial.
6. Drop-wise, add 0,5 ml per vial of freezing media II for cells and mix.
7. Rapidly place the vials to -80°C. Transfer cells to -150^{0}C or liquid nitrogen the next day for long-term storage.

NOTE: If the treatment with 0,5 mM EDTA seems insufficient to remove the cells from the dish use a cell lifter to gently scrape the colonies from the dish instead of breaking the colonies into too small pieces by triturating harshly with a pipette.