Culturing conditions of histone H1 knock-out cell lines derived from WAe001 H1.

1. Coat culture dish with rh-Laminin-521 diluted 1:40 in D-PBS/Ca^{2+}/Mg^{2+}. Incubate for two hours at 37°C, or well-sealed for up to 5 days at 4°C (for volumes required refer to Table 1).
2. Pre-warm Accutase enzyme in a 37°C water bath (for volumes required refer to Table 2).
3. Aspirate the conditioned medium from the growing cells of the culture dish.
4. Rinse the culture dish once with D-PBS\$Ca^{2+}$\$Mg^{2+}$ (for volumes required refer Table 2).
5. Add pre-warmed Accutase to the culture dish.
6. Incubate at 37°C, 5% CO_2 for 5 minutes or until some gaps are visible within the cell colonies.
7. Gently wash the cells of the dish and pipette the solution up and down 5-10 times to generate a single cell suspension.
8. Transfer the cell suspension to a conical tube containing Essential 8 Medium to dilute and neutralize Accutase (for volumes required refer to Table 2).
9. Wash the culture dish with 1-2 ml of Essential 8 Medium to collect the remaining cells. Combine with the cell suspension in the conical tube.
10. Centrifuge the cells at 200 *x g* for 4 minutes, RT.
11. Discard the supernatant, flick the tube 3-5 times to loosen the pellet, and resuspend the cells by pipetting up and down 5-10 times in Essential 8 Medium (for volumes required refer to Table 2).
12. Determine the cell viability using your preferred method (automated cell counter or manual).
13. Calculate the volume of cell suspension required to seed cells at the desired density (see Table 3).
14. Transfer the calculated volume of cells to a pre-coated dish containing the recommended volume of Essential 8 Medium supplemented with Rock inhibitor (see Table 3). To distribute cells evenly on the surface, move the dish back-and-forth and side-to-side in several quick motions. Avoid swirling the cells and moving in circles.
15. Incubate the cells at 37°C, 5% CO_2.
16. Essential 8 Medium must be changed daily.
17. To maintain optimum health of the cultures, cells should be passaged when reaching 60 – 85% confluency.
18. Cells are cryopreserved in Essential 8 Medium containing 10% (v/v) DMSO and supplemented with Rock inhibitor.
19. Thawed cells must be supplemented with Rock inhibitor to promote survival.

Table 1: *Volumes of diluted rhLaminin-521 required for different culture plates and dishes.*

culture dish (surface area)	volume of 1:40 rh-Laminin-521
6-well (10 cm^2)	2 mL
12 well (4 cm^2)	0.8 mL
24 well (2 cm^2)	0.4 mL
35 mm (10 cm^2)	2 mL
60 mm (21 cm^2)	4 mL
100 mm (55 cm^2)	12 mL

Table 2: *Volumes of reagents needed for cell passaging into different culture plates and dishes.*

culture dish (surface area)	D-PBS\$Ca^{2+}$\$Mg^{2+}$ for washing	Accutase	Essential 8 Medium*	Essential 8 Medium**
6-well (10 cm^2)	2 mL	1 mL	3 mL	2 mL
12 well (4 cm^2)	1 mL	0.4 mL	1.2 mL	1 mL
24 well (2 cm^2)	0.5 mL	0.2 mL	0.6 mL	0.5 mL
35 mm (10 cm^2)	2 mL	1 mL	3 mL	2 mL
60 mm (21 cm^2)	4 mL	2 mL	6 mL	4 mL
120 mm (55 cm^2)	12 mL	6 mL	18 mL	12 mL

*for neutralization; **for resuspension

Table 3*: Recommended cell seeding densities and volumes of medium required for different culture plates and dishes.*

culture dish (surface area)	12,500 cells/cm^2	25,000 cells/cm^2	Essential 8 Medium
6-well (10 cm^2)	125,000	250,000	2 mL
12 well (4 cm^2)	50,000	100,000	1 mL
24 well (2 cm^2)	25,000	50,000	0.5 mL
35 mm (10 cm^2)	125,000	250,000	2 mL
60 mm (21 cm^2)	250,000	500,000	4 mL
100 mm (55 cm^2)	750,000	1,500,000	12 mL

Time to reach confluency: 4–5 days for 12,500 cells/cm^2 seeding density and 3–4 days for 25,000 cells/cm^2 seeding density.