

L1 cell culture and Wnt3a conditioned Media Protocol

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L1 control and L1/Wnt3a cells are from Chris Hughes lab

Growth medium: The base medium for this cell line is Dulbecco's Modified Eagle's Medium. To make the complete growth medium, add the following components to the base medium: fetal bovine serum (omega scientific, cat# FB-02) to a final concentration of 10%.

Subculturing Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Protocol for control/Wnt3a conditioned medium:

1. Split the cells 1:15-20 in 10 ml culture medium in 10 cm petri dishes and let the cells grow for 4 days (approximately to confluency). (DO NOT need to change media)
2. Take off the medium and sterile filter. This is the first batch of medium.
3. Add 10 ml fresh culture medium and culture for another 3 days.
4. Take off the medium and sterile filter. This is the second batch of medium. Discard the cells, because they will be overgrown by now.
5. Mix the first batch and second batch of medium 1:1. This is the L Cell conditioned medium. It is stable at 4°C and can be frozen. .
6. Test the conditioned medium by Luciferase Assay in different dilutions; choose a best working concentration for subsequent experiments.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:10 is recommended (why is this different

from above? This is for L1 cell subculture, starting with more cells and change media more often comparing with making conditioned media)

Medium Renewal: Every 2 to 3 days.

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO.

Storage temperature: liquid nitrogen vapor phase.