NEUROMICS

Protocol for Human Pericytes in T25 Flask

Recommended Products

- Pericyte Growth Media
  - Growth Media designed for pericyte growth. Contains the following two components: 495 ml of DMEM (high glucose) and 5 ml of Pericyte-Growth supplement.

- Smooth Coat Solution – SC300
  - Biocompatible complex of extracellular matrix binding solution

OR

- AlphaBioCoat Solution – AC001
  - Premium Smooth Coat Solution. Biocompatible complex of extracellular matrix binding solution with growth factors. Ideal for culturing cells from frozen.

- Cell Detachment Solution – ADF001
  - Contains protease and collagenase activities in an isotonic, phosphate buffer solution with EDTA to detach primary cells and cell lines

- 1X Phosphate Buffer Solution - PBS300

Shipping

Proliferating culture in T25 flask.

Handling of Arriving Cells

When you receive the cells, leave the flask in 37°C CO_2 incubator for 1 hour first, and then replace the transport medium with fresh Full medium. Let the cells grow for 24 hours before subculture.

Note: Handling human derived products is potentially bioharzadous. Although each cell strain tests negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination.

SUBCULTURE PROTOCOL

1. *Coating T25 flasks. Add 2 ml Smooth Coat Solution (SC300) into 3- T25 flask and ensure entire interior surface is coated with solution. After 30 minutes, dispose of Smooth Coat Solution by aspiration. Gently rinse and aspirate flask with phosphate buffer Solution (PBS300). The flask is now ready for use (no need for overnight incubation when coated with SC300). Add fresh media to flask, if color changes from pink to yellow, discard the media, and add fresh media to each flask.

2. Inspect to make sure Flask is at 90% confluence, if not remove transport media, and add 5ml of fresh media to the flask. Place flask in 37°C incubator until cells are at 90% confluence. Change media every 2 days.

3. If flask is at 90% confluence, aspirate transport media from flask.

4. Rinse T25 flask containing cells with 5 ml 1XPBS (cat#PBS300).

5. Gently aspirate out the PBS after rinsing, and discard.

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6. Add 2ml of RT trypsin/ EDTA or Cell Detachement Solution (ADF001) to T25 flask containing cells (ensure entire interior surface is cover).

7. Place T25 flask containing cells into 37°C incubator for 1 or 2 minutes (cells will normally come off of the surface within 1 or 2 minutes).

8. Suspend the cells with 10ml of Pericyte-Growth medium (PGB001) and transfer equally into 2 pre-coated T25 flasks (the cells are now at a subculture ratio of 1:2).

    a. Future passages can have a subculture ratio of 1:3, once the cells are more stable.
        i. Suspend the cells with 15ml of Pericyte-Growth medium and transfer equally into 3 pre-coated T25 flasks

9. There is no need to spin cells during subculture.

10. Proliferating cell culture: Pericyte-Growth medium should be changed every 2 days. The cells normally become confluent within 7 days (when split at a 1:3 ratio)

11. Use Pericyte- Basal media (PGB002) containing 0.5% FBS to induce quiescent cells (after 18-24 hours).

Note: Should any issues arise while using our cells, our team is here to help troubleshoot any issues. Our cells are backed by our one-time replacement or refund policy. Our recommended protocol including recommended products must be used to be eligible for replacement or refund. Cells that have been refrozen are no longer eligible for refund or replacement.

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