

ReNcell VM Immortalized Cell Line**CATALOG NUMBER:** SCC008**LOT NUMBER:****QUANTITY:** >1 X 10⁶ viable cells upon thawing.

DESCRIPTION:

ReNcell VM is an immortalized human neural progenitor cell line with the ability to readily differentiate into neurons and glial cells. ReNcell VM was derived from the ventral mesencephalon region of a human fetal brain tissue. Immortalized by retroviral transduction with the v-myc oncogene, this cell line grows rapidly as a monolayer on laminin with a doubling time of 20-30 hours. Karyotype analyses indicate that the ReNcell VM retains a normal diploid karyotype in culture even after prolonged passage (>45 passages). ReNcell VM was developed by the ReNeuron Group plc, a biotech company that specializes in using human somatic stem cells for therapeutics. In experiments performed by the ReNeuron Group plc, ReNcell VM can be differentiated *in vitro* to a high level of human dopaminergic neurons. Neurons differentiated from ReNcell VM have furthermore been shown to be electrophysiologically active. ReNcell VM may be used for a variety of research applications such as studies of neurotoxicity, neurogenesis, electrophysiology, neurotransmitter and receptor functions.

For Research Use Only; not for use in diagnostic procedures. ReNcell Immortalized Cells have been isolated in a legal and ethical manner compliant with local informed consent procedures.

KIT COMPONENTS:

1. > 1x10⁶ viable ReNcell VM cells: (CHEMICON® Cat. No. SCC008) derived from 10-week human ventral mesencephalon brain tissue, cryopreserved. Store in liquid nitrogen.

CHARACTERIZATION OF CELLS:

Each lot of ReNcell VM cells has been validated for high level of expression of Nestin and Sox 2 and for their self-renewal and multi-lineage differentiation capacities (please refer to datasheet figures). Cells also display normal karyotype as assessed by chromosome spread and tested negative for mycoplasma.

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005)
2. ReNcell NSC Freezing Medium (CHEMICON® Cat. No. SCM007)
3. Basic fibroblast growth factor (bFGF; FGF-2; Specific Activity ≥ 2 X 10⁶ Units/mg. CHEMICON® Cat. No. GF003)
4. Epidermal growth factor (EGF; Specific Activity ≥ 1 x 10⁷ Units/mg; CHEMICON® Cat. No. GF001)
5. Laminin (Sigma Cat. No. L-2020)
6. DMEM/F12 w/o HEPES, w/ L-Glutamine (CHEMICON® Cat. No. DF-042-B)
7. Accutase™ (CHEMICON® Cat. No. SCR005)

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8. Tissue culture-ware
9. Phosphate-Buffered Saline (1X PBS) (CHEMICON® Cat. No. BSS-1005-B)
10. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
11. Blocking Solution (5% normal donkey serum, 0.3% Triton X-100 in 1X PBS)
12. Primary and secondary antibodies
13. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution
14. Anti-fading mounting solution (DABCO/PVA)
15. Hemacytometer
16. Microscope

STORAGE:

When stored at the recommended storage conditions (liquid nitrogen), ReNcell VM cells are stable up to the expiration date. Do not expose to elevated temperatures. Discard any remaining reagents after the expiration date. We recommend that the cells be used within ten passages.

PREPARATION OF COATED FLASKS:

We recommend coating tissue culture plastic- or glasswares that are used to culture ReNcell VM cells with laminin. Tissue culture flasks should be coated on the same day that the ReNcell VM cells are thawed from liquid nitrogen or on the same day that the cells need to be passage. The following procedure is recommended:

1. Thaw the laminin in the morning at 2-8°C. Dilute laminin with DMEM/F12 (CHEMICON® Cat. No. DF-042-B) to 20 µg/mL.
2. Add enough of the diluted laminin solution to cover the whole surface of the tissue culture-ware. Use 3 mL volume for 6-cm plates and 6.5 mL volume for 10-cm plates and T75 flasks. Incubate in a 37°C, 5% CO₂ incubator for at least 4 hours.
3. Just before use, aspirate the laminin solution in the coated flasks and rinse the flasks once with 1X PBS.
4. Prepare the Complete ReNcell NSC Medium by adding 20 ng/mL FGF-2 and 20 ng/mL EGF (final concentrations) to ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005).
5. Add 10 mL of the freshly made Complete ReNcell NSC Medium to the laminin-coated T75 flasks. Incubate in a 37°C, 5% CO₂ incubator. The laminin-coated flasks are now ready to receive the cells.

THAWING OF CELLS:

1. Do not thaw the cells until the recommended medium and appropriately coated laminin plasticware and/or glassware are on hand.
2. Remove the vial of ReNcell VM cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful to not introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005) (pre-warmed to 37°C) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of medium at once to the cells. This may result in decreased cell viability due to osmotic shock.**

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6. Gently mix the cell suspension by slow pipeting up and down twice. Be careful to not introduce any bubbles.
IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 4-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 5 mL of ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005) (pre-warmed to 37°C) containing freshly added 20 ng/mL FGF-2 and 20 ng/mL EGF.
Note: FGF-2 and EGF should always be added fresh to the ReNcell NSC Maintenance Medium.
10. Plate the cell mixture onto the laminin-coated T75 tissue culture flask that was pre-incubated in the 37°C incubator. The laminin coated T75 flask should already have 10 mL of Complete ReNcell Neural Stem Cell Medium (i.e. ReNcell NSC Maintenance Medium containing 20 ng/mL FGF-2 and 20 ng/mL EGF).
11. Incubate the cells at 37°C in a 5% CO₂ humidified incubator.
12. The next day, exchange the medium with fresh ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005) (pre-warmed to 37°C) containing 20 ng/mL FGF-2 and 20 ng/mL EGF. Exchange with fresh medium containing FGF-2 and EGF every other day thereafter.
13. When the cells are approximately 80% confluent, they can be dissociated with Accutase™ and passaged or alternatively frozen for later use.

SUBCULTURING:

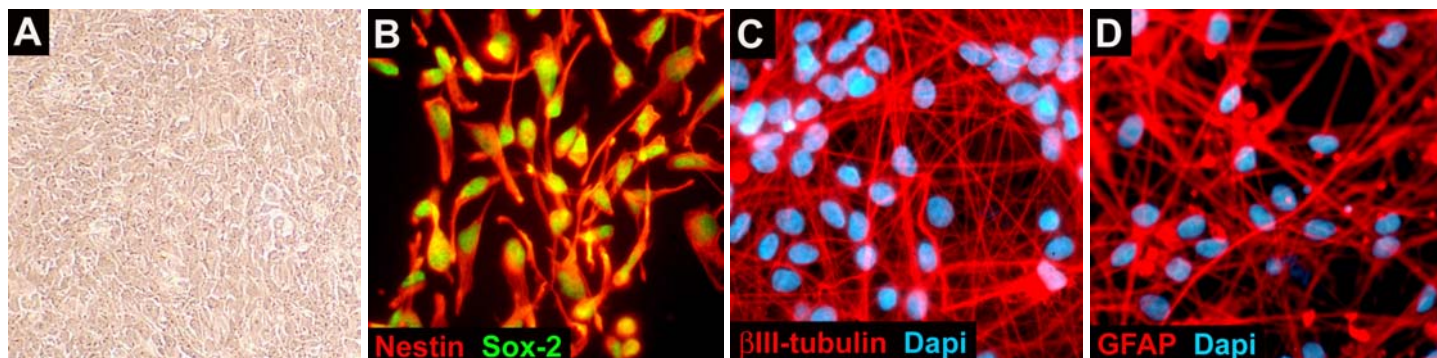
1. Prepare fresh laminin-coated flasks (refer to Preparation of Coated Flasks).
2. Carefully remove the medium from the laminin-coated T75 flasks containing the confluent layer of ReNcell VM cells.
3. Rinse the flask once with 1X PBS. **Note:** Add the PBS slowly from the side to avoid detaching the cells.
4. Aspirate the PBS.
5. Apply 3-5 mL of Accutase™ and incubate in a 37°C incubator for 3-5 minutes.
6. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
7. Apply 5 mL of ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005) (pre-warmed to 37°C) to the flask.
8. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
9. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
10. Discard the supernatant.
11. Apply 2 mL of ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005) containing 20 ng/mL FGF-2 and 20 ng/mL EGF to the conical tube and resuspend the cells thoroughly. **Note:** Do not vortex the cells.
12. Count the number of cells using a hemacytometer.
13. Plate the cells to the desired density into the appropriate fresh laminin-coated flasks, plates or wells in ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005) containing 20 ng/mL FGF-2 and 20 ng/mL EGF. We typically plated the cells at ~1.5 million cells on laminin coated T75 flasks.
14. The next day, exchange the medium with fresh ReNcell NSC Maintenance Medium (CHEMICON® Cat. No. SCM005) containing 20 ng/mL FGF-2 and 20 ng/mL EGF. Exchange with fresh medium containing FGF-2 and EGF every other day thereafter. The cells should be ready for passaging or harvesting 2 to 3 days after this step.

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DIFFERENTIATION (FOR 8-WELL CHAMBER SLIDES):

1. The 8-well chamber slides should be coated with 20 $\mu\text{g}/\text{mL}$ laminin (please refer to the section on Preparation of Coated Flasks).
2. Plate out 30,000 cells per well into an appropriately coated 8-well chamber slide in ReNcell NSC Maintenance Medium (CHEMICON[®] Cat. No. SCM005) containing 20 ng/mL FGF-2 and 20 ng/mL EGF. Total volume per well = 0.5 – 0.75 mL. At this density the cells should be ~50% - 60% confluent by the next day. **Note:** *To prevent overgrowth of the cells by the end of the two-week differentiation protocol, it is best to avoid plating too many cells.*
3. The next day, initiate differentiation by removing the medium from each well and replacing with fresh ReNcell NSC Maintenance Medium (CHEMICON[®] Cat. No. SCM005) that does not contain FGF-2 and EGF. **Note:** *Differentiation is initiated by withdrawing the growth factors so FGF or EGF should not be added to the basal medium.*
4. Replace with fresh ReNcell NSC Maintenance Medium (CHEMICON[®] Cat. No. SCM005) every 2-3 days for two weeks. **Note:** *It is important that FGF or EGF not be present in the basal medium.*
5. After two weeks, the cells can be fixed with 4% paraformaldehyde and stained with the desired antibodies.

CHARACTERIZATION OF ReNcell VM IMMORTALIZED CELL LINE (SCC008):

ReNcell VM cells (CHEMICON[®] Cat. No. SCC008) are grown as monolayers (A) and express NSC markers, Nestin (B, red) and Sox-2 (B, green). ReNcell VM cells are able to differentiate into neurons (β III-tubulin; C) and glial cells (GFAP; D).

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Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered worldwide
www.chemicon.com • custserv@chemicon.com • techserv@chemicon.com