ReNcell™ CX Human Neural Progenitor Cell Line & Culture Media Kit

Kit

Cat. # SCC009 **Pack size: 1 Kit**

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NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS

Data Sheet

Description

ReNcell™ CX Human Neural Progneitor Cell Line (SCC007): ReNcell™ CX is an immortalized human neural progenitor cell line with the ability to readily differentiate into neurons and glial cells. ReNcell™ CX was derived from the cortical region of human fetal brain tissue. Immortalized by retroviral transduction with the c-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™ CX retains a normal diploid karyotype in culture even after prolonged passage (>45 passages). ReNcell™ CX was developed by the ReNeuron Group plc, a biotech company that specializes in using human somatic stem cells for therapeutics. ReNcell™ CX may be used for a variety of research applications such as studies of neurotoxicity, neurogenesis, electrophysiology, neurotransmitter and receptor functions. Each lot of ReNcell™ CX 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.
ReNcell™ NSC Maintenance Medium (SCM005):ReNcell™ Neural Stem Cell (NSC) Maintenance Medium is a defined serum-free, growth factor-free medium that has been optimized for the growth and in vitro differentiation of ReNcell™ immortalized human neural progenitor cells. When used in conjunction with FGF and EGF, the maintenance medium will allow for the proliferation of ReNcell™ immortalized VM and CX neural stem cells. Withdrawal of the growth factors from ReNcell™ NSC Maintenance Medium will result in the spontaneous differentiation of ReNcell™ immortalized neural progenitor cells.
Composition: ReNcell™ NSC Maintenance Medium contains DMEM/F12 w/o HEPES, L-glutamine, human serum albumin, human transferrin, putrescine dihydrochloride, human recombinant insulin, L-thyroxine, tri-iodo-thyronine, progesterone, sodium selenite, heparin, and corticosterone.

ReNcell™ NSC Freezing Medium (SCM007): ReNcell™ NSC Freezing Medium is qualified for use with ReNcell™ immortalized human neural progenitor cell lines, CX (Millipore Catalog No. SCC007) and VM (Millipore Catalog No. SCC008) cultured in serum-free conditions with ReNcell™ NSC Maintenance Medium (Millipore Catalog No. SCM005). The optimized formulation allows for consistent cryopreservation and high viability upon thawing and plating.
Composition: Serum-free formulation. Contains 10% DMSO

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. This product contains genetically modified organisms.

KIT CONTENTS:

- ReNcell™ CX Human Neural Progenitor Cell Line (SCC007): $\geq$ 1 X 10^6 viable cells upon thawing. Derived from 14-week human cortical brain tissue, cryopreserved.
- ReNcell™ NSC Maintenance Medium (SCM005), 500 mL
- ReNcell™ NSC Freezing Medium (SCM007), 50 mL

STORAGE:

CELLS: When stored at the recommended storage conditions (liquid nitrogen), ReNcell™ CX 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.

MAINTENANCE MEDIUM: Store at -20ºC until ready to use. Upon thawing, this media should be stored at 2-8ºC and given a 1-month expiration dating.

FREEZING MEDIUM: Store at -20ºC. Refer to lot expiration date on label.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Rev.1.0/2018-04-10/SCC009/AN

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. Basic fibroblast growth factor (bFGF; FGF-2; Specific Activity > 2 X 106 Units/mg. Millipore Cat. No. GF003)
2. Epidermal growth factor (EGF; Specific Activity > 1 x 107 Units/mg; Millipore Cat. No. GF001)
3. Laminin (Sigma Cat. No. L-2020)
4. DMEM/F12 w/o HEPES, w/ L-Glutamine (Millipore Cat. No. DF-042-B)
5. Accutase™ (Millipore Cat. No. SCR005)
6. Tissue culture-ware
7. Phosphate-Buffered Saline (1X PBS) (Millipore Cat. No. BSS-1005-B)
8. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)
9. Blocking Solution (5% normal donkey serum, 0.3% Triton X-100 in 1X PBS)
10. Primary and secondary antibodies
11. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS Solution
12. Anti-fading mounting solution (DABCO/PVA)
13. Hemacytometer
14. Microscope

PREPARATION OF COATED FLASKS:

We recommend coating tissue culture plastic- or glasswares that are used to culture ReNcell™ CX cells with laminin. Tissue culture flasks should be coated on the same day that the ReNcell™ CX 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 2-8ºC. Dilute laminin with DMEM/F12 (Millipore 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 3mL volume for 6-cm plates and 6.5mL volume for 10-cm plates and T75 flasks. Incubate in a 37ºC, 5% CO_2 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 20ng/mL FGF-2 and 20ng/mL EGF (final concentrations) to ReNcell™ NSC Maintenance Medium (Millipore Cat. No. SCM005).
5. Add 10mL of the freshly made Complete ReNcell™ NSC Medium to the laminin-coated T75 flasks. Incubate in a 37ºC, 5% CO_2 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™ CX cells from liquid nitrogen and incubate in a 37ºC 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 2mL pipette to transfer the cells to a sterile 15mL conical tube. Be careful to not introduce any bubbles during the transfer process.
5. Using a 10mL pipette, slowly add dropwise 9 mL of ReNcell™ NSC Maintenance Medium (Millipore Cat. No. SCM005) (pre-warmed to 37ºC) to the 15mL 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.**
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 5mL of ReNcell™ NSC Maintenance Medium (Millipore Cat. No. SCM005) (pre-warmed to 37ºC) containing freshly added 20ng/mL FGF-2 and 20ng/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 37ºC incubator. The laminin coated T75 flask should already have 10mL of Complete ReNcell™ NSC Medium (i.e. ReNcell™ NSC Maintenance Medium containing 20ng/mL FGF-2 and 20 ng/mL EGF).
11. Incubate the cells at 37ºC in a 5% CO_2 humidified incubator.
12. The next day, exchange the medium with fresh ReNcell™ NSC Maintenance Medium (Millipore Cat. No. SCM005) (pre-warmed to 37ºC) containing 20ng/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™ CX 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 37ºC 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 5mL of ReNcell™ NSC Maintenance Medium (Millipore Cat. No. SCM005) (pre-warmed to 37ºC) to the flask.
8. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15mL conical tube.
9. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
10. Discard the supernatant.
11. Apply 2mL of ReNcell™ NSC Maintenance Medium (Millipore Cat. No. SCM005) containing 20ng/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 (Millipore 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 (Millipore 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.

DIFFERENTIATION (FOR 8-WELL CHAMBER SLIDES):

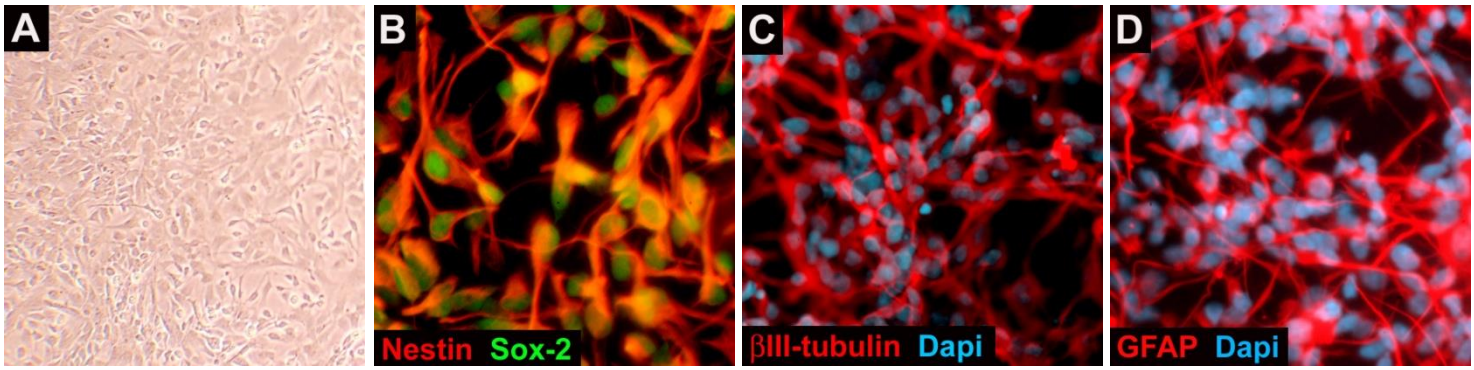
1. The 8-well chamber slides should be coated with 20 µg/mL laminin (please refer to the section on Preparation of Coated Flasks).
2. Plate out 30,000 cells per well into an appropriately coated 8-well chamber slide in ReNcell™ NSC Maintenance Medium (Millipore Cat. No. SCM005) containing 20 ng/mL FGF-2 and 20 ng/mL EGF. Total volume per well = 0.5 – 0.75mL. 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 (Millipore 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 (Millipore 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.

CRYOPRESERVATION OF RENCELL™ CX using ReNcell™ Freezing Medium (SCM007)

1. Thaw cell culture freezing medium completely and mix well by gently swirling bottle. Keep freezing medium on ice during use.
2. Cells to be frozen should be in late log phase growth.
3. Monolayers will need to be dissociated. After dissociation, cells are resuspended in ReNcell™ NSC Maintenance Medium (Catalog No. SCM005) and counted to determine viability and number.
4. Centrifuge cells at 1300 rpm for 3 min. Remove the medium above the pellet.
5. Resuspend the cells in cell culture freezing medium at a concentration of ~4 X 10^6 cells/mL. Freeze 1 mL of cells/vial. After the cells have been resuspended and aliquoted into appropriate cryogenic storage vials, they can be placed in a freezing container and the normal freeze down procedure should be started within five minutes.
6. Cells must be stored at or below -80ºC. For long term storage the cells should be stored in ultra-low temperature freezer (-150ºC), or in liquid nitrogen (-196ºC).
7. Thawing of cryopreserved cells should be as follows:

   a. Thaw cells quickly in a 37ºC water bath.
   b. Dilute one vial of cells into 10 mL of prewarmed ReNcell™ NSC Maintenance Medium.
   c. Gently mix the cells in the growth medium.
   d. Gently pellet the cells and remove the medium above the pellet.
   e. Resuspend the cells in ReNcell™ NSC Maintenance Medium with the appropriate concentration of FGF-2 and EGF and plate into the appropriate vessel.

CHARACTERIZATION OF ReNcell™ CX IMMORTALIZED CELL LINE (SCC007):



ReNcell™ CX cells (Millipore Cat. No. SCC007) are grown as monolayers (A) and express NSC markers, Nestin (B, red) and Sox-2 (B, green). ReNcell™ CX cells are able to differentiate into neurons (□III-tubulin; C) and glial cells (GFAP; D). For color images please go to www.millipore.com.

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