

ReproNSC Medium Kit: Instructions for Use

Overview

This protocol describes procedures for culturing human NSCs (Neural Stem Cells) in ReproNSC Medium.

Required Materials

PRODUCT DESCRIPTION	CAT. NO.	Storage Conditions
ReproNSC Medium Kit “ <ul style="list-style-type: none"> • ReproNSC Basal Medium • ReproNSC Supplement A • ReproNSC Supplement B 	00-0077 01-021-100 05-0051 05-0052	See label: 2-8 °C -20 °C -20 °C
Corning® Matrigel® hESC Qualified Matrix, *LDEV-free, Stem Cell qualified	Corning 354277	As indicated
DMEM, high glucose, pyruvate	ThermoFisher 11995081	As indicated
StemPro™ Accutase™ Cell Dissociation Reagent	ThermoFisher A1110501	As indicated

Store all required materials according to the manufacturer’s recommendations.

ReproNSC Medium Kit Intended Use

This product is intended for Research Use Only (RUO). Not intended for human or animal diagnostic or therapeutic use.

Handling upon Receipt

The ReprONSC Medium Kit is shipped in two boxes in one package: one on dry ice, and one at 4 °C with ice packs. Upon receipt, check the integrity of the packages, including the presence of dry ice. (Notify ReproCELL (info@reprocell.com) if the package has been compromised.)

Store the individual components as specified on the label or the table above.

Culturing Protocol

All procedures should be performed in a biological safety cabinet using proper sterile technique to minimize the possibility of contamination. Wear PPE (lab coat, glasses, gloves) as indicated by institution biosafety procedures.

Coating Cell Culture Vessel with Matrigel®

Cell culture vessels should be coated one day before or on the day of plating of the NSCs. Read general use instructions in manufactures documentation. Work should be performed in a biological safety cabinet to preserve sterility.

1. Pre-chill pipette tips and dishes at 4 °C.
2. Thaw aliquot (typically 270-350 µL of Corning® Matrigel® at 4 °C. Transfer the aliquot to ice.
3. Prepare a 25 mL aliquot of cold DMEM in a 50 mL conical tube and keep on ice.
4. Transfer 1 mL cold DMEM to the tube with the Matrigel. Mix up and down several times. Transfer the solution to the 50 mL tube of DMEM. Mix up and down several times. Store on ice.
5. Immediately coat pre-chilled culture dishes with Matrigel/DMEM solution. Suggested coating volume: 120-150 µL/cm².

Vessel Type	Amount of DMEM/Matrigel solution
96-well plate	50 µL/well
4- or 24 well plate	250 µL/well
35 mm dish	1.5 mL
60 mm dish	2.5 mL

6. Distribute the coating matrix solution evenly and incubate at room temperature (15-25 °C) for at least 1 hr.
7. Coated dishes can be used immediately or stored at 4 °C for up to 7 days.
8. Pre-warm vessels at 37 °C before use.
9. Aspirate Matrigel solution immediately before seeding. Do not let surface dry.

Preparation of ReprONSC Complete Medium

1. Thaw frozen supplements at room temperature.

2. Mix 50 mL **ReproNSC Maintenance Medium**, 1 mL **Supplement A**, and 10 µL **Supplement B** to give **ReproNSC Complete Medium**. Store unused components at 4 °C and use within 7 days. **ReproNSC Complete Medium** can be stored at 2-8 °C and should be used within 7-10 days.
3. Pre-warm medium at 37 °C before use.

Passaging and plating NSCs in ReproNSC Complete Medium

1. Prepare Matrigel-coated culture dishes as described earlier. Warm at 37 °C. Pre-warm Accutase, DMEM, and **ReproNSC Complete Medium**.
2. Remove medium from confluent dish of NSCs. Add Accutase immediately at approximately 100 µL/cm².
3. Incubate at 37 °C for 3-5 min until cells detach. Use a P1000 pipette to wash off cells from dish and transfer to a 15 mL conical tube. Rinse dish with equal volume of DMEM and transfer to same tube.
4. Centrifuge at 400 x g for 5 min.
5. Aspirate supernatant and resuspend pellet in 2-4 mL **ReproNSC Complete Medium**.
6. Perform a cell count if necessary.
7. Aspirate Matrigel solution from prepared dishes. Add **ReproNSC Complete Medium** to cover bottom of dish.
8. Transfer cell suspension to dish at a density of 4-6x10⁴ cells/cm².

Alternatively, cells can be plated at a ratio of 1:4 in the same size vessel as previously used. Cells from a 60 mm dish can be plated in a 100 mm dish.

9. Distribute cells evenly. Incubate cells at 37 °C, 5% CO₂ in a humidity-controlled cell culture incubator.
10. Change medium every other day until NSCs are confluent.

REPROCELL USA Inc

9000 Virginia Manor Road
Suite 207
Beltsville, MD 20705
USA

Tel: +1 301 470 3362
Email: info-us@reprocell.com

REPROCELL Europe Ltd

Thomson Pavilion, Todd Campus
West of Scotland Science Park
Acre Road
Glasgow G20 0XA
UK

Tel: +44 (0)141 465 3460
Email: info-emea@reprocell.com

REPROCELL India Ltd

3-1-135/1A, CNR Complex
Mallapur
Hyderabad 500 076
Telangana
India

Tel: +44 (0)141 465 3460
Email: Bhargavi.Gurram@reprocell.com

REPROCELL Inc (Japan)

MetLife Shin-yokohama 381, Bldg. 9F
3-8-11, Shin-yokohama
Kohoku-ku, Yokohama
Kanagawa 222-0033
Japan

Tel: +81 45 475 3887
Email: info-asia@reprocell.com