User Guide

Quick-Neuron™ Cholinergic - Maintenance Medium

Catalog Number: CH-MM

Introduction

Quick-Neuron™ Cholinergic - Maintenance Medium may be used for the long-term maintenance of human pluripotent stem cell-derived cholinergic neurons following differentiation as outlined in the Quick-Neuron™ Cholinergic - SeV Kit, mRNA Kit, and Human iPSC-derived Neurons user guides. Quick-Neuron™ Cholinergic differentiated cell cultures display typical neurite outgrowth and express a variety of neuronal markers, such as the pan-neuronal marker tubulin beta 3 class III (TUBB3) and the cholinergic neuron marker choline acetyltransferase (ChAT). When handled and maintained according to the instructions in this user guide, cholinergic neurons are viable long-term and are suitable for a variety of characterization and neurotoxicity assays.

Scale: The Quick-Neuron™ Cholinergic - Maintenance Medium provides sufficient medium for 4 wells of a 24-well plate for up to 2 weeks.

Related Products: Quick-Neuron™ Cholinergic - SeV Kit, Catalog Number: CH-SeV
Quick-Neuron™ Cholinergic - mRNA Kit, Catalog Number: CH-mRNA
Quick-Neuron™ Cholinergic - Human iPSC-derived Neurons, Catalog Number: CH-SeV-CW

Kit Contents

Upon receipt, store the reagents at the temperatures indicated in the table below. All reagents are shipped on dry ice.

Reagents	Volume	Storage
Component N1	830 µl	-20°C or -80°C
Component B	16 µl	-20°C or -80°C
Component P	14 µl	-20°C or -80°C

Required Consumables

Item	Vendor	Catalog Number
DMEM/F12	ThermoFisher	21331020
Neurobasal Medium	ThermoFisher	21103049
Glutamax (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122

Conditions of Use

This product is for research use only. It is not approved for use in humans or for therapeutic or diagnostic use.

Technical Support

For technical support, please contact us at cs@elixirgensci.com or call +1 (443) 869-5420 (M-F 9 am-5 pm EST).

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Base Media Preparation

Medium N1

1. Prepare Medium N1 using the reagents listed in the table below.
    - Thaw Component N1 at 4°C overnight or 30 minutes on ice.
    - All other reagents should be warmed at room temperature for 20-30 minutes.

Medium N1 Reagents	Volume
DMEM/F12	8 ml
Neurobasal Medium	8 ml
200 mM Glutamax (100x)	83 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	167 µl
Component N1	500 µl

2. Store Medium N1 for up to 2 weeks at 4°C.
    - The leftover Component N1 can be discarded or saved for another use.

First Week

Medium N1(BP)

1. Prepare Medium N1(BP) using the reagents listed in the table below.
    - Thaw Component B at 4°C overnight or 30 minutes on ice. Spin down before use.
    - All other reagents should be warmed at room temperature for 20-30 minutes.

Medium N1(BP) Reagents	Volume
Medium N1	7 ml
Component B	7 µl
Component P	3.5 µl

2. Save the leftover Component B at 4°C.
    - The leftover Component P can be discarded or saved for another use.
3. Warm Medium N1(BP) at room temperature for 20-30 minutes until it no longer feels cold.
4. Pipet out most of the old medium, but not completely (i.e., just enough to cover the surface of the well), from each well using a P1000 pipettor and add 800 µl Medium N1(B) along the wall of the well very slowly.
5. Incubate the cultures at 37°C, 5% CO_2 for 2 days.
6. For subsequent medium changes, pipet out half (400 µl) of the old medium from each well using a P1000 pipettor and add 400 µl Medium N1(BP).
7. Repeat Step 6 every 2-3 days such as on Monday, Wednesday, and Friday for 1 week.

Second Week

Medium N1(B)

1. Prepare fresh Medium N1(B) using the reagents listed in the table below.
    - Warm Medium N1 at room temperature for 30 minutes.
    - Place Component B on ice. Spin down before use.

Medium N1(B) Reagents	Volume
Medium N1	7 ml
Component B	7 µl

2. Warm Medium N1(B) at room temperature for 20-30 minutes until it no longer feels cold.
3. Pipet out most of the old medium, but not completely (i.e., just enough to cover the surface of the well), from each well using a P1000 pipettor and add 800 µl Medium N1(B) along the wall of the well very slowly.
4. Incubate the cultures at 37°C, 5% CO_2 for 2 days.
5. For subsequent medium changes, pipet out half (400 µl) of the old medium from each well using a P1000 pipettor and add 400 µl Medium N1(B).
6. Repeat Step 5 every 2-3 days such as on Monday, Wednesday, and Friday for 1 week.