User Guide

Quick-Neuron™ Motor - Maintenance Medium

Catalog Number: MT-MM

Introduction

Quick-Neuron™ Motor - 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™ Motor - SeV Kit and Human iPSC-derived Neurons user guides. Quick-Neuron™ Motor 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), the cholinergic marker choline acetyl-transferase (ChAT), and the homeobox transcription factor HB9 expressed in motor neurons. When handled and maintained according to the instructions in this user guide, motor neurons are viable long-term and are suitable for a variety of characterization and neurotoxicity assays.

Scale: The Quick-Neuron™ Motor - Maintenance Medium provides sufficient medium for 4 wells of a 24-well plate, 1 well of a 6-well plate, or 16 wells of a 96-well plate for up to 2 weeks.

Related Products: Quick-Neuron™ Motor - SeV Kit, Catalog Number: MT-SeV
Quick-Neuron™ Motor - Human iPSC-derived Neurons, Catalog Number: MT-SeV-CW

Contents

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

Contents	Volume	Storage	Thaw
Component N1	830 µl	-20°C or -80°C	On ice or 4°C
Component B	20 µl	-20°C or -80°C	On ice or 4°C
Component P	50 µl	-20°C or -80°C	Room temperature

Condition 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 refer to the FAQ on our website.
You may also contact us at cs@elixirgensci.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

Required Consumables

Item	Vendor	Catalog Number
DMEM/F12	ThermoFisher	21331020
Neurobasal Medium	ThermoFisher	21103049
GlutaMAX	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122

Preparation

Medium N1(B)

1. Prepare Medium N1(B) using the reagents listed in the table below.
    - Thaw Components N1 and B for 20-30 minutes at the temperature indicated in the “Contents” table on page 1.
    - Warm all other reagents at room temperature for 20-30 minutes.
    - Tap each Component tube 3 times and then briefly spin all tubes down before use.
    - Keep Medium N1(B), and any subsequent media made with it, protected from light.
    - Store Medium N1(B) for up to 2 weeks at 4°C.
    - Leftover Component N1 can be discarded or saved at 4°C for up to two weeks.

Reagents	Volume
DMEM/F12	7.2 ml
Neurobasal Medium	7.2 ml
GlutaMAX	75 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	150 µl
Component N1	450 µl
Component B	15 µl

First Week

1. Prepare Medium N1(BP) using the reagents listed in the table below.
    - Thaw Component P for 20-30 minutes at the temperature indicated in the “Contents” table on page 1.
    - Warm all other reagents at room temperature for 20-30 minutes.
    - Tap the Component P tube 3 times and then briefly spin it down before use.
    - Store Medium N1(BP) for up to 2 weeks at 4°C.
    - Leftover Component P can be saved at 4°C.

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

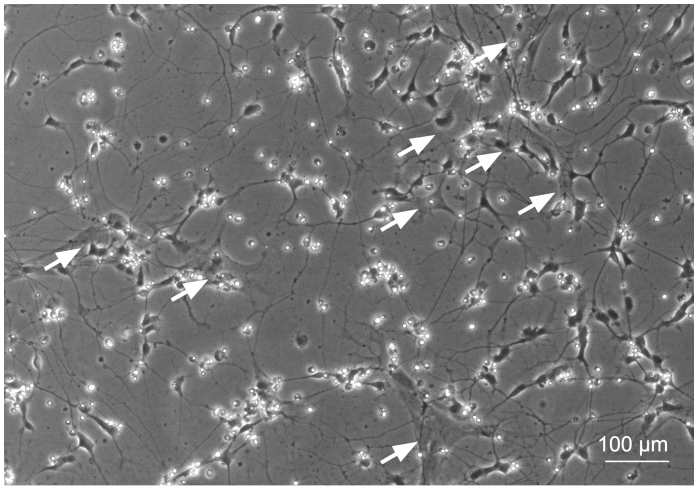
2. Pipet out most of the old medium, but not completely (i.e., just enough to cover the surface of the well), from each well and very slowly along the wall of the well, add Medium N1(BP) according to the following table.

	Required volume per well		
Reagents	**6-well plate**	**24-well plate**	**96-well plate**
Medium N1(BP)	2 ml	800 µl	150 µl

3. Incubate the cultures at 37°C, 5% CO_2 for 2 days.
4. For subsequent medium changes, pipet out half (see volumes in the table above) of the old medium from each well using and replace with an equal volume of room temperature Medium N1(BP).
5. Repeat Step 4 every 2-3 days such as on Monday, Wednesday, and Friday for 1 week.

Second Week

1. Warm Medium N1(B) at room temperature for 20-30 minutes until it no longer feels cold.
   Note: If there is an outgrowth of non-neuronal flat cells in the culture (as seen marked by arrows in the sample image below) users should continue using Medium N1(BP) in the second week, following the instructions to prepare Medium N1(BP) provided in the “First Week” .



2. Pipet out most of the old medium, but not completely (i.e., just enough to cover the surface of the well), from each well and very slowly along the wall of the well, add Medium N1(B) according to the following table.

	Required volume per well		
Reagents	**6-well plate**	**24-well plate**	**96-well plate**
Medium N1(B)	2 ml	800 µl	150 µl

3. Incubate the cultures at 37°C, 5% CO_2 for 2 days.
4. For subsequent medium changes, pipet out half (see volumes in the table above) of the old medium from each well using and replace with an equal volume of room temperature Medium N1(B).
5. Repeat Step 4 every 2-3 days such as on Monday, Wednesday, and Friday for 1 week.