User Guide

Ricoh Biosciences, Inc.

Quick-Neuron™ GABAergic - Maintenance Medium

Catalog Number: GA-MM

Introduction

Quick-Neuron™ GABAergic - Maintenance Medium may be used for the long-term maintenance of human pluripotent stem cell-derived GABAergic neurons following differentiation as outlined in the Quick-Neuron™ GABAergic - mRNA Kit and Human iPSC-derived Neurons user guides. Quick-Neuron™ GABAergic 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 GABAergic marker glutamic acid decarboxylase (GAD67). When handled and maintained according to the instructions in this user guide, GABAergic neurons are viable long-term and are suitable for a variety of characterization and neurotoxicity assays.

Scale: The Quick-Neuron™ GABAergic - 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™ GABAergic - mRNA Kit, Catalog Number: GA-mRNA
Quick-Neuron™ GABAergic - Human iPSC-derived Neurons, Catalog Number: GA-mRNA-HC-CW50065

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 N	840 µl	-20°C or -80°C	On ice or 4°C
Component G2	60 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@biosciences.ricoh.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 N(G2)

1. Prepare Medium N(G2) using the reagents listed in the table below.
    - Thaw each component 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.
    - Briefly spin down all Components before use.
    - Keep Medium N(G2), and any subsequent media made with it, protected from light.
    - Store Medium N(G2) for up to 2 weeks at 4°C.
    - Leftover Components N and G2 can be discarded or saved at 4°C for up to two weeks.

Reagents	Volume
DMEM/F12	6 ml
Neurobasal Medium	6 ml
GlutaMAX	63 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	126 µl
Component N	391 µl
Component G2	12.6 µl

First Week

1. Prepare Medium N(G2P) 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.
    - Briefly spin down Component P before use.
    - Store Medium N(G2P) for up to 2 weeks at 4°C.
    - Leftover Component P can be saved at 4°C.

Reagents	Volume
Medium N(G2)	5.2 ml
Component P	5.2 µl

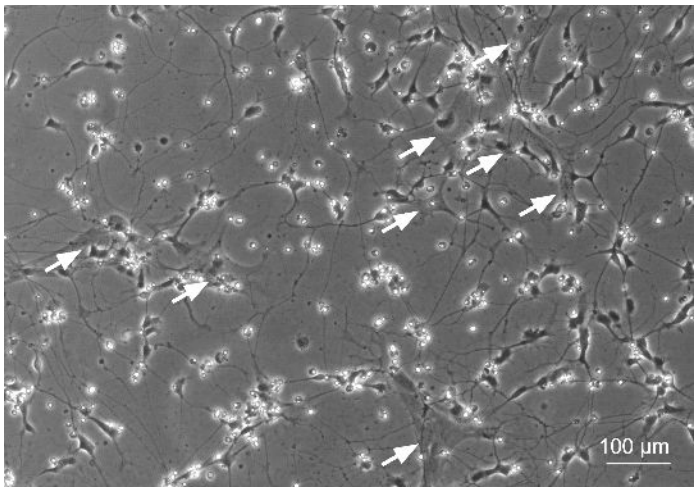
2. Pipet out half of the old medium from each well using and very slowly along the wall of the well, add room temperature Medium N(G2P) according to the following table.

Reagents	Required volume per well		
	6-well plate	24-well plate	96-well plate
Medium N(G2P)	1 ml	400 µl	75 µl

3. Incubate the cultures at 37°C, 5% CO_2 for 2 days.
4. Repeat Steps 2-3 every 2-3 days such as on Monday, Wednesday, and Friday for 1 week.

Second Week

1. Warm Medium N(G2) 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 N(G2P) in the second week, following the instructions to prepare Medium N(G2P) 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 N(G2) according to the following table.

Reagents	Required volume per well		
	6-well plate	24-well plate	96-well plate
Medium N(G2)	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 and replace with an equal volume of room temperature Medium N(G2).
5. Repeat Step 4 every 2-3 days such as on Monday, Wednesday, and Friday for 1 week.