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Quick-Neuron™ Mixed Maintenance Medium

Catalog Number: EXGS-QNMM

User Manual

This kit (EXGS-QNMM) contains 1 set of reagents for use with a total of 4 wells of a 24-well plate for 2 weeks

Table of Contents:

I. Introduction

Thank you for purchasing the Quick-Neuron™ Mixed Maintenance Medium. This kit is intended for use after completing the protocol of the Quick-Neuron™ Mixed - SeV Complete kit (Cat. Number: EXGS-QNMSV). The Quick-Neuron™ Mixed kit allows users to differentiate human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) into a population of mixed neurons within 10 days. The population includes tubulin beta 3 Class III (TUBB3, a pan-neuronal marker), choline acetyltransferase (ChAT, a cholinergic neuron marker), tyrosine hydroxylase (TH, a dopaminergic neuron marker), parvalbumin (PVALB, a GABAergic neuron marker), vesicular glutamate transporter 1 (vGLUT1, a glutamatergic neuron marker), and tryptophan hydroxylase 2 (TPH2, a serotonergic neuron marker)-positive neurons. To extend your culture of mixed neurons beyond 10 days, we recommend culturing the neurons with Quick-Neuron™ Mixed Maintenance Medium. The kit provides sufficient neuron maintenance medium for 4 wells of a 24-well plate for up to 2 weeks (Catalog Number: EXGS-QNMM).

II. Kit Contents

Upon receipt of this kit, immediately store all reagents at their proper storage temperatures as described in the table below. All reagents are shipped on dry ice.

List of Components

Reagents	Amount	Storage Conditions
Component N	840 µl	-20 °C
Compoentn B	16 µl	-20 °C
Component K	25 µl	-20 °C

NOTE: RETINOIC ACID SUPPLEMENTS

- Retinoic acid (RA) is known to induce the differentiation of forebrain neurons (Reference 1).
- Our immunocytochemistry and quantitative reverse transcription PCR results demonstrated that addition of RA had no significant influence on the population of mixed neurons using the Quick-Neuron™ Mixed - SeV Complete Kit.
- This kit separately provides RA as Component K. Users may supplement Medium ND with Component K at a concentration of 1 µl Component K per 1 ml medium.

III. Additional Materials Required

The following materials are needed but not supplied with this kit:

- DMEM/F12 (e.g., ThermoFisher, Catalog Number: 21331-020)
- Neurobasal (e.g., ThermoFisher, Catalog Number: 21103049)
- Glutamax (100x) (e.g., ThermoFisher, Catalog Number: 35050061)
- Penicillin-Streptomycin (e.g., ThermoFisher, Catalog Number: 15140-122)

IV. Pre-Protocol Preparation

- Prepare a neural maturation medium by mixing following reagents after thawing Componen N at 4°C overnight. The medium is called Medium N and stable for up to 2 weeks when stored at 4°C.

No.	Reagent	Volume
1	DMEM/F12	12 ml
2	Neurobasal	12 ml
3	200 mM Glutamax (100x)	125 µl
4	Penicillin-Streptomycin (10000 units/ml; 100x)	250 µl
5	Component N	775 µl

V. Protocol

Day 1

1. Warm Medium N at room temperature for 20-30 minutes.
2. Thaw Component B on ice for 20-30 minutes.
3. Take 15 ml Medium N into a tube and add 15 µl Component B to it. Mix them well. This medium is referred to as Medium N(B) and is stable for up to 2 weeks at 4°C.
4. (If adding retinoic acid is desired) thaw Component K at room temperature for 20-30 minutes. Add 15 µl Component K to 15 ml Medium N(B). This medium is referred to as Medium N(BK).
5. Pipet out all old medium from each well using a P1000 pipettor and add 800 µl Medium N(B) or Medium N(BK) to it.
6. Incubate the cultures at 37°C, 5% CO_2 for 2 days.

Day 3-14

1. Warm Medium N(B) or Medium N(BK) at room temperature for 20-30 minutes.
2. Pipet out half (400 µl) of the old medium from each well using a P1000 pipettor and add 800 µl Medium N(B) or Medium N(BK) to it.
3. Incubate the cultures at 37°C, 5% CO2 for 2 days.
4. Repeat steps 1-3 every 2 days.

IV. Appendix: Literature Reference

1. Cunningham TJ and Duester G (2015). Mechanisms of retinoic acid signalling and its roles in organ and limb development. Nat Rev Mol Cell Biol 16, 110–123.

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Category	Product	Request Quote (Catalog number)		
		SeV Complete Kit	mRNA Complete Kit	Maintenance Medium
Quick-Tissue™ Series Differentiation Kit (mainly for 4 wells of 24-well plate)	Quick-Endothelium™ Vascular with Optional Drug Selection		$399 (EXGS-QEV)	$229 (EXGS-QEVM)
	Quick-Trilineage™ Differentiation Kit*	$549 (EXGS-Q3D)		
	Quick-Neuron™ Mixed	$349 (EXGS-QNMSV)		$129 (EXGS-QNMM)
	Quick-Neuron™ Cholinergic	$349 (EXGS-QNCSV)	$299 (EXGS-QNC)	$129 (EXGS-QNCM)
		$999 (EXGS-QNCSV96)**		
	Quick-Neuron™ Dopaminergic	$399 (EXGS-QNDSV)	$399 (EXGS-QND)	$169 (EXGS-QNDM)
	Quick-Neuron™ GABAergic	$399 (EXGS-QNGSV)	$399 (EXGS-QNG)	$149 (EXGS-QNGM)
	Quick-Muscle™ Skeletal	$349 (EXGS-QMSSV)		$129 (EXGS-QMSM)
	Quick-Hepatocyte™	$499 (EXGS-QHSV)		$149 (EXGS-QHM)
	Quick-miniBrain™	$999 (EXGS-QMBSVMR)		

15% off for 3 or more kit purchases per order

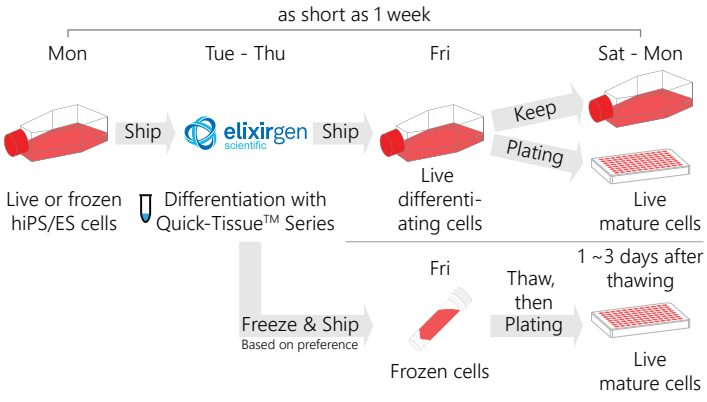
* This kit provides 2 wells per each tissue (total 6 wells of 24-well plate)

** This kit is for 48 wells of a 96-well microplate format

Category	Product	Size	Request Quote (Catalog number)
Quick-Tissue™ Series Differentiation Support	Quick-Tissue™ Mesendoderm Booster	8 wells of 24-well plate	$99 (EXGS-QTMB)
	Quick-Tissue™ Adaptation Kit	a 35-mm dish or 1 well of 6-well plate	$169 (EXGS-QTA1)
	New product coming soon!		
Reagents for Maintaining Undifferentiated Stem Cells	Ajinomoto StemFit® Basic02	500 mL	Ask (EXGS-ASB02)
	Nippi iMatrix-511 silk	175 µg x 6 tubes	Ask (EXGS-NI511S)
	Nippi iMatrix-511	175 µg x 6 tubes	$690 (EXGS-NI511)

15% off for 3 or more kit purchases per order

Quick-Tissue™ Stem Cell Differentiation Services



Elixirgen Scientific provides pluripotent stem cell differentiation services with the world's fastest turnaround time. Customers can simply ship live iPS/ES cells in a T-25 flask and will receive live or fronzen cells in a week (express service) or two weeks (regular service). Contact services@elixirgenscientific.com for more details to customize for your project.
Currently Elixirgen Scientific offers all tissue types from kits for cell differentiation services. More tissue types are coming soon!