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Quick-Endothelium™ Vascular Maintenance Medium

Catalog Number: EXGS-QEVM

User Manual

This kit (EXGS-QEVM) contains 1 set of reagents to proliferate CD31-positive cells derived from 4 wells in a 24-well plate for 2 weeks

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I. Introduction

Thank you for purchasing the Quick-Endothelium™ Vascular Maintenance Medium. This kit is intended for use after completing the protocol of the Quick-Endothelium™ Vascular kit (Catalog Number: EXGS-QEV). The Quick-Endothelium™ Vascular kit allows users to differentiate human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), into CD31-positive vascular endothelial cells within one week. To extend the culture of vascular endothelial cells beyond one week, we recommend culturing the endothelial cells with Quick-Endothelium™ Vascular Maintenance Medium. The kit provides sufficient medium to proliferate cultures of endothelial cells derived from 4 wells of a 24-well plate for up to 2 weeks (Cat. Number: EXGS-QEVM).

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 J1	16.5 µl	-20 °C
Component J2	33 µl	-20 °C
Solution D1	1 ml	-20 °C
Coating Material A	15.7 µl x 2	-20 °C

- This kit contains iMatrix-511 silk (Nippi, Inc.).

III. Additional Materials Required

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

- 35 mm culture dish or 6-well tissue-culture-treated polystyrene plate
- Minimum Essential Media (MEM) α, no nucleosides (e.g., ThermoFisher Scientific, Catalog Number: 12561-056)
- KnockOut Serum Replacement (KSR, ThermoFisher Scientific, Catalog Number: 10828-010)
- Sodium Pyruvate (100 mM) (e.g., ThermoFisher Scientific, Catalog Number: 11360-070)
- MEM Non-Essential Amino Acids Solution (100X) (e.g., ThermoFisher Scientific, Catalog Number: 11140-050)
- Glutamax (100x) (e.g., ThermoFisher, Catalog Number: 35050061)
- Penicillin-Streptomycin (e.g., ThermoFisher, Catalog Number: 15140-122)
- Phosphate-buffered saline (PBS without Ca^{++} Mg^{++})
- β-mercaptoethanol (β-ME, ThermoFisher Scientific, Catalog Number: 21985-023)
- ROCK inhibitor Y27632 (e.g., Selleckchem Catalog Number: s1049)
- Dimethyl sulfoxide (DMSO; e.g., Sigma-Aldrich, Catalog Number: D8418)
- CD31-positive vascular endothelial cell cultures prepared using the Quick-Endothelium™ Vascular kit (Catalog Number: EXGS-QEV)

IV. Pre-Protocol Preparation

- Prepare a 10 mM β-ME stock solution in PBS to mix with Medium E(J1J2) on Day 1. Preparation steps are as follows:
  - Mix 80 µl 55 mM β-ME with 360 µl PBS.
  - Sterile filter the mixture.
  - Store at 4°C
- Prepare a vascular endothelial cell maintenance medium by mixing following reagents. The medium is called Medium E(J1J2) and stable for up to 2 weeks when stored at 4°C.

No.	Reagent	Volume
1	Minimum Essential Media (MEM) α, no nucleosides	28.8 ml
2	KnockOut Serum Replacement	1.6 ml
3	Sodium Pyruvate (100 mM)	320 µl
4	MEM Non-Essential Amino Acids Solution (100X)	320 µl
5	200 mM Glutamax (100x)	320 µl
6	Penicillin-Streptomycin (10000 units/ml; 100x)	320 µl
7	10 mM β-ME	320 µl
8	Component J1	16 µl
9	Component J2	32 µl

- Prepare a 10 mM ROCK inhibitor Y27632 stock solution in DMSO to prepare Medium iE(J1J2) on Day 0 and Day 8. Preparation steps are as follows:
  - Dissolve 10 mg ROCK inhibitor Y27632 in 3.1225 ml DMSO.
  - Make aliquots of a convenient volume (e.g., 100 µl) and store at -20°C.
- We do not recommend additional freeze-thaw cycles of any reagents.
- Taking 4x and/or 10x images of cultures every day (or even after every medium change) is a good way to monitor your experiment.

IV. Protocol

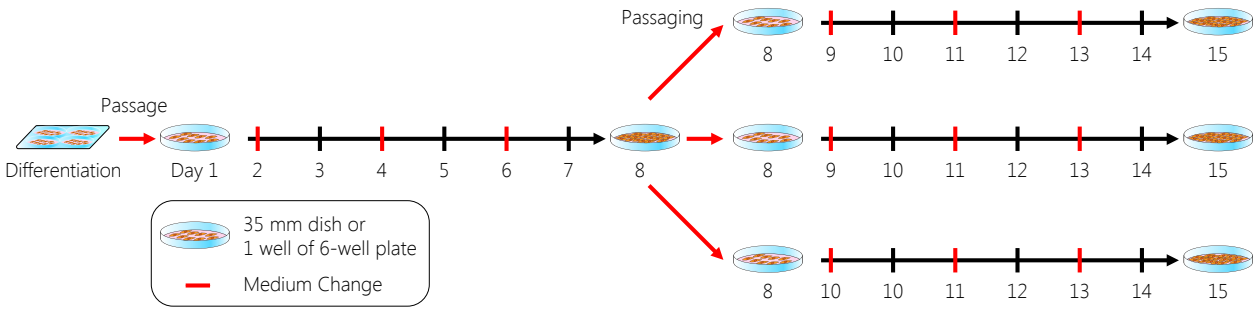

Figure 1: Schematic representation of maintenance of Vascular Endothelial cells differentiated from hPSCs using Quick-Endothelium™ Vascular kit in Quick-Endothelium™ Vascular Maintenance Medium

Day 1 - Passaging

Cells can be passaged if they are 80-100% confluent. After first passage the new cultures may take 7 days to reach near to confluency again.

New Plate Preparation

1. Start thawing Solution D1 and warm Medium E(J1J2) at room temperature. Make sure that Solution D1 and Medium E(J1J2) are at room temperature for at least 1 hour before use.
2. Thaw Coating Material A on ice for 20-30 minutes (or at 4°C overnight one day before Day 0).
3. Take 2 ml ice-cold PBS into a new 15 ml conical tube and add 6.6 µl Coating Material A to it. Mix them well. Keep the rest Coating Material A at 4°C for its use at Day 8.
4. Add 2 ml diluted Coating Material A to one 35 mm culture dish or one well of a 6-well plate.
5. Incubate the dish or plate at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 0).
6. Aspirate the supernatant from the dish or well and add 2 ml PBS to it.
7. Incubate the dish or plate at 37°C, 5% CO_2 until user's endothelial cells are ready for plating.

Plating

1. Pipet out old medium from each vascular endothelial cell culture using a P1000 pipettor and add 500 µl PBS to it.
2. Rock the plate 3 times, pipet out PBS from each well using a P1000 pipettor, and add 75 µl Solution D1 to it. Keep the rest of Solution D1 at 4°C for its use on Day 8.
3. Incubate the cultures at 37°C, 5% CO_2 for 5-7 minutes.
4. Take 2.0 ml Medium E(J1J2) into a new 15 ml conical tube and add 2.0 µl 10 mM ROCK inhibitor to it. Mix them well. The medium is referred to as Medium iE(J1J2).
5. Carefully pipet out Solution D1 from each well using a P1000 pipettor and add 250 µl Medium iE(J1J2) to it.
6. Disperse the medium over the well bottom surface by pipetting 8-15 times to detach cells.
7. Collect the cell suspension from each well in a tube.
8. Add Medium iE(J1J2) to bring up the volume to 1.5 ml.
9. Aspirate PBS from the coated dish or well and add 1.5 ml cell suspension to it.
10. Incubate the culture at 37°C, 5% CO_2 overnight.

Day 2-6 - Feeding

1. Warm Medium E(J1J2) at room temperature for 20-30 minutes.
2. Pipet out the old medium from the dish or well using a P1000 pipettor and add 1.5 ml Medium E(J1J2) to it.
3. Incubate the culture at 37°C, 5% CO_2 for 2 days.
4. Repeat steps 1-3 every 2 days until Day 8.

Day 8 - Passaging

New Plate Preparation

1. Thaw Coating Material A on ice for 20-30 minutes (or at 4°C overnight one day before Day 0).
2. Take 6.5 ml ice-cold PBS into a new 15 ml conical tube and add 21.5 µl Coating Material A to it. Mix them well.
3. Add 2 ml diluted Coating Material A to three 35 mm culture dish or three wells of a 6-well plate.
4. Incubate the dishes or plate at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 0).
5. Aspirate the supernatant from each dish or well and add 2 ml PBS to it.
6. Incubate the dishes or plate at 37°C, 5% CO_2 until user's endothelial cells are ready for plating.

Plating

1. Warm Solution D1 and Medium E(J1J2) at room temperature for 20-30 minutes.
2. Pipet out old medium from the endothelial culture using a P1000 pipettor and add 500 µl PBS to it.
3. Rock the plate 3 times, pipet out PBS from the culture using a P1000 pipettor, and add 300 µl Solution D1 to it.
4. Incubate the culture at 37°C, 5% CO_2 for 5-7 minutes.
5. Take 5 ml Medium E(J1J2) into a new 15 ml conical tube and add 5 µl 10 mM ROCK inhibitor to it. Mix them well.
6. Carefully pipet out Solution D1 from the culture using a P1000 pipettor and add 1 ml Medium iE(J1J2) to it.
7. Disperse the medium over the well bottom surface by pipetting 8-15 times to detach cells.
8. Collect the cell suspension in a tube.
9. Add Medium iE(J1J2) to bring up the volume to 4.5 ml.
10. Aspirate PBS from each coated dish or well and add 1.5 ml cell suspension to it.
11. Incubate the cultures at 37°C, 5% CO_2 overnight.

Day 9-13 - Feeding

1. Warm Medium E(J1J2) at room temperature for 20-30 minutes.
2. Pipet out the old medium using from each dish or well using a P1000 pipettor and add 1.5 ml Medium E(J1J2) to it.
3. Incubate the cultures at 37°C, 5% CO_2 for 2 days.
4. Repeat steps 1-3 every 2 days until Day 15.

Day 15 - Ready for Assay

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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

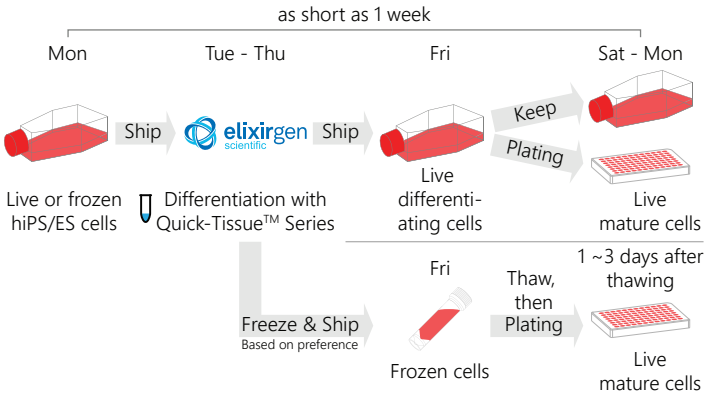
* This kit provides 2 wells per each tissue (total 6 wells of 24-well plate)

** 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!