User Guide

Quick-Endothelium™ Vascular - Maintenance Medium

Catalog Number: VE-MM

Introduction

Quick-Endothelium™ Vascular - Maintenance Medium may be used for the long-term maintenance of human pluripotent stem cell-derived vascular endothelial cells following differentiation as outlined in the Quick-Endothelium™ Vascular - mRNA Kit user guide. Quick-Endothelium™ Vascular differentiated cell cultures display typical endothelial morphology and capillary tube formation, and express endothelial cell markers, such as cluster of differentiation 31 (CD31) and von Willebrand factor (vWF) as well as the tight junction marker Claudin-5 (CLDN5). When handled and maintained according to the instructions in this user guide, vascular endothelial cells are viable long-term and are suitable for a variety of characterization and toxicity assays.

Scale: The Quick-Endothelium™ Vascular - Maintenance Medium provides for the expansion and maintenance of up to 4 wells of a 24-well plate to approximately 3 wells of a 6-well plate over the course of 2 weeks.

Related Products: Quick-Endothelium™ Vascular - mRNA Kit (Small), Catalog Number: VE-mRNA-S

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
Coating Material A	15.7 µl x 2	-20°C or -80°C

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

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 9am-5pm EST).

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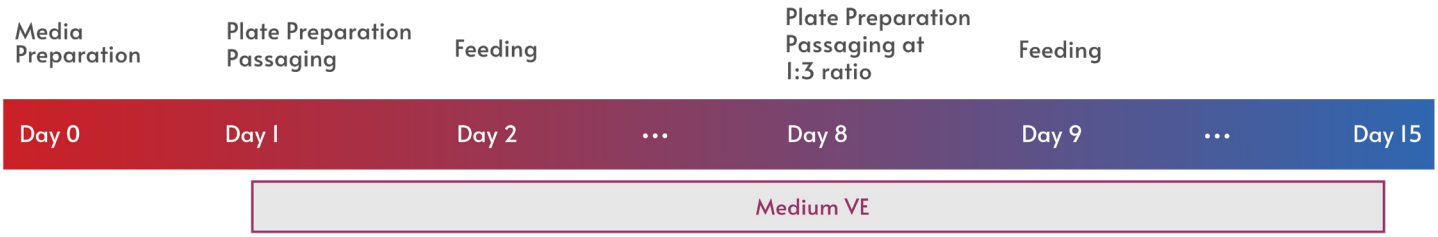
Required Consumables

Item	Vendor	Catalog Number
(Optional) 24-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-740
(Optional) 6-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)*	Fisher Scientific	07-200-80
Minimum Essential Media (MEM) α, no nucleosides	ThermoFisher	12561056
KnockOut Serum Replacement (KSR)	ThermoFisher	10828010
Sodium Pyruvate (100mM)	ThermoFisher	11360070
MEM Non-Essential Amino Acids Solution (100X)	ThermoFisher	11140050
Glutamax (100x)	ThermoFisher	35050061
Penicillin-Streptomycin	ThermoFisher	15140122
TrypLE Select Enzyme (1X)**	ThermoFisher	12563011
0.02% EDTA in DPBS	Sigma-Aldrich	E8008-100ML
Phosphate-buffered saline (without Ca^{++} Mg^{++})	ThermoFisher	20012050
β-mercaptoethanol (β-ME)	ThermoFisher	21985023
ROCK inhibitor Y27632	Selleckchem	S1049
SB431542	Selleckchem	S1067
VEGF-165	Peprotech	100-20
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418
Nuclease-free H_20	Fisher Scientific	AM9937
50% Glycerol solution, sterile	Fisher Scientific	50-841-704
30% Bovine Serum Albumin (BSA) solution	Sigma-Aldrich	A9576

*Can be substituted with 35-mm culture dishes.
**Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

Workflow



Note: Depending on the inital number of wells (1-4) from the 24-well plate and the confluence of the cells in those wells, the day of passaging will vary and may be earlier or later than Day 8 indicated in the workflow above. We recommend a 1:3 split when passaging the cells. You may need to passage more than once during a 15 day period. Do not allow the cells to become more than 80-90% confluent.

Media Preparation

10 mM ROCK inhibitor Y27632 (iROCK)

1. Dissolve 10 mg ROCK inhibitor Y27632 in 3.12 ml DMSO.
2. Make aliquots of a convenient volume (e.g., 100 µl).
3. This solution, hereafter referred to as iROCK, can be stored at -20°C.

0.5X TrypLE Select with EDTA (Solution D1)*

1. Mix 1 ml TrypLE Select Enzyme (1X) with 1 ml 0.02% EDTA in DPBS.
2. This mixture, hereafter referred to as Solution D1, can be stored at 4°C for 2 weeks.

*Can be substituted with our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

10 mM β-ME stock solution in PBS (10 mM β-ME)

1. Mix 80 µl 55 mM β-ME with 360 µl PBS.
2. Sterile filter the mixture, hereafter referred to as 10mM β-ME, and store at 4°C.

10 mM SB431542

1. Dissolve 10 mg SB431542 in 2.60 ml DMSO.
2. Make aliquots of a convenient volume (e.g., 100 µl).
3. Store at -80°C for up to two years.

20 µg/ml VEGF-165

1. Dissolve 2 µg VEGF-165 in 20 µl of 0.1% BSA prepared with nuclease-free H_2O.
2. Add 60 µl 0.1% BSA prepared with PBS.
3. Add 20 µl of 50% Glycerol solution.
4. Make aliquots of a convenient volume (e.g., 20 µl).
5. This mixture, hereafter referred to as 20 µg/ml VEGF-165, can be stored at -20°C.

Medium VE

1. Prepare Medium VE using the reagents listed in the table below.
    - Thaw 20 µg/ml VEGF-165 on ice for 20-30 minutes.
    - All other reagents should be warmed at room temperature for 20-30 minutes.

Medium VE Reagents	Volume
Minimum Essential Media (MEM) α, no nucleosides	28.8 ml
KnockOut Serum Replacement	1.6 ml
Sodium Pyruvate (100 mM)	320 µl
MEM Non-Essential Amino Acids Solution (100X)	320 µl
200 mM Glutamax (100x)	320 µl
Penicillin-Streptomycin (10000 units/ml; 100x)	320 µl
10 mM β-ME	320 µl
20 µg/ml VEGF-165	16 µl
10 mM SB431542 (1,000x)	32 µl

2. Store Medium VE for up to 2 weeks at 4°C.

Day 1

~4 hr

IMPORTANT! Cells should be passaged if cultures reach 80-90% confluency. After passaging, the new cultures may take 7 days to reach confluency again.

Plate Preparation

1. Prepare diluted Coating Material A by mixing together the following components in a 15 ml conical tube.
    - Thaw Coating Material A on ice for 20-30 minutes (or at 4°C overnight one day before Day 1).
    - Make sure chilled PBS is used for this mixture.
    - Keep the rest of Coating Material A at 4°C.

Diluted Coating Material A Reagents	Volume
Coating Material A	6.6 µl
Chilled PBS	2 ml

2. Add 2 ml diluted Coating Material A to 1 well of a 6-well plate.
3. Incubate the plate at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 1).
4. Aspirate the supernatant from the well and add 500 µl PBS.
5. Incubate the plate at 37°C, 5% CO_2 until the cells are ready for plating.

Plating

1. Prepare Medium iVE by mixing together the following components in a 15 ml conical tube.
    - Warm Medium VE, iROCK, and Solution D1 at room temperature for 1 hour.

Medium iVE Reagents	Volume
Medium VE	2 ml
iROCK	2 µl

2. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
3. Pipet the old medium out from each of up to 4 wells of vascular endothelial cell culture (on a 24-well plate) using P1000 pipettor and add 500 µl PBS.
4. Rock the plate 3 times, pipet out PBS from each well using a P1000 pipettor, and add 75 µl of the cell dissociation reagent Solution D1. Keep the rest of Solution D1 at 4°C for its use on subsequent passages.
5. Incubate the cultures at 37°C, 5% CO_2 for 5 minutes. If all the cells are not rounded under a microscope, incubate at 37°C, 5% CO_2 for up to 5 more minutes in 1-2 minute increments.
6. Carefully pipet out Solution D1 from each well using a P1000 pipettor and add 250 µl Medium iVE.
7. Disperse the medium over the well bottom surface by pipetting 8-15 times to detach cells.
8. Collect the cell suspension from each well into a tube.
9. Add Medium iVE to bring up the volume to 1.5 ml.
10. Aspirate PBS from the coated well and add 1.5 ml cell suspension.
11. Incubate the culture at 37°C, 5% CO_2 overnight.

Days 2-8

~1 hr

Maintenance

1. Warm Medium VE at room temperature for 20-30 minutes.
2. Pipet out the old medium from the well using a P1000 pipettor and add 2 ml Medium VE.
3. Incubate the culture at 37°C, 5% CO_2 for 2-3 days.
4. Repeat Steps 1-3 every 2-3 days until cells are ready for passaging.

Note: If the cell confluency is more than 60% use 3 ml medium per well.

Day 8 (Or When Cells Reach 80-90% Confluency)

~4 hr

Plate Preparation

1. Prepare diluted Coating Material A by mixing together the following components in a 15 ml conical tube.
    - Thaw Coating Material A on ice for 20-30 minutes (or at 4°C overnight one day before Day 8).
    - Make sure chilled PBS is used for this mixture.

Diluted Coating Material A Reagents	Volume
Coating Material A	21.5 µl
Chilled PBS	6.5 ml

2. Add 2 ml diluted Coating Material A to 3 wells of a 6-well plate.
3. Incubate the plate at 37°C, 5% CO_2 for 2 hours (or 4°C overnight one day before Day 8).
4. Aspirate the supernatant from the well and add 500 µl PBS.
5. Incubate the plate at 37°C, 5% CO_2 until the cells are ready for plating.

Plating

1. Prepare Medium iVE by mixing together the following components in a 15 ml conical tube.
    - Warm Medium VE, iROCK, and Solution D1 at room temperature for 1 hour.

Medium iVE Reagents	Volume
Medium VE	5 ml
iROCK	5 µl

2. Tap the Solution D1 tube 5 times with a finger and centrifuge at maximum speed for 1 minute.
3. Pipet out old medium from the well using a P1000 pipettor and add 500 µl PBS.
4. Rock the plate 3 times, pipet out PBS from each well using a P1000 pipettor, and add 300 µl of the cell dissociation reagent Solution D1.
5. Incubate the cultures at 37°C, 5% CO2 for 5 minutes. If all the cells are not rounded under a microscope, incubate at 37°C, 5% CO2 for up to 5 more minutes in 1-2 minute increments.
6. Carefully pipet out Solution D1 from the well using a P1000 pipettor and add 1 ml Medium iVE to it.
7. Disperse the medium over the well bottom surface by pipetting 8-15 times to detach cells.
8. Collect the cell suspension from the well into a tube.
9. Add Medium iVE to bring up the volume to 4.5 ml.
10. Aspirate PBS from each coated well and add 1.5 ml cell suspension to each well.
11. Incubate the culture at 37°C, 5% CO2 overnight.

Continuing Culture

1. Continue Maintenance as in the “Maintenance” section on the previous page.
2. Passage as needed when the cells reach 80-90% confluency following the instructions above. We recommend a 1:3 split as above.
3. Make more Medium VE following the instructions above as needed for expansion and maintenance of the cells.