User Guide

Quick-Endothelium™ Vascular - Human iPSC-derived Cells

Catalog Numbers: VE-mRNA-CW50065, VE-mRNA-CW10149, VE-mRNA-CW20300, VE-mRNA-CW50023, VE-mRNA-CW70067, VE-mRNA-CW50025, VE-mRNA-CW50113, VE-mRNA-CW50114, VE-mRNA-CW50115, VE-mRNA-CW50137, VE-mRNA-CW50147, VE-mRNA-CW60130, VE-mRNA-CW60231, VE-mRNA-CW60236, VE-mRNA-CW20026, VE-mRNA-CW20090, or VE-mRNA-CW10130

Introduction

Elixirgen Scientific's proprietary transcription factor-based stem cell differentiation method uses synthetic mRNAs to produce highly pure populations of vascular endothelial cells (VECs) without a genetic footprint. Quick-Endothelium™ Vascular - Human iPSC-derived Cells display morphology similar to human umbilical vein endothelial cells (HUVECs) and express a variety of endothelial cell markers, such as platelet and endothelial cell adhesion molecule 1 (PECAM1, also known as CD31), claudin 5 (CLDN5), and von willebrand factor (VWF). When handled and maintained according to the instructions in this user guide, vascular endothelial cells are suitable for a variety of characterization and functional assays.

Scale: Quick- Endothelium™ Vascular- Human iPSC-derived Cells are available in two sizes: (Small) 1 million viable cryopreserved cells and (Large) 5 x 1 million viable cryopreserved cells.

Related Products: Quick-Endothelium™ Vascular - mRNA Kit, Catalog Number: VE-mRNA
Quick-Endothelium™ Vascular - Maintenance Medium, Catalog Number: VE-MM

Kit Contents

Upon receipt, immediately store the items at the indicated temperatures. Be especially careful to keep the frozen cells on dry ice until placing them in liquid nitrogen and avoid any temperature fluctuation and slight thawing.

Contents	Amount (Small Size)	Amount (Large Size)	Storage
Cryopreserved cells	>1 million viable cells, (1 vial, 500 µl)	5 x >1 million viable cells, (5 vials, 5 x 500 µl)	Liquid nitrogen

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 contact us at cs@elixirgensci.com or call +1 (443) 869-5420 (M-F 9am-5pm EST).

Required Consumables

Item	Vendor	Catalog Number
(Optional) 6-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-80
(Optional) 24-well tissue-culture-treated polystyrene plate (e.g., Corning Costar Flat Bottom Cell Culture Plates)	Fisher Scientific	07-200-740
iMatrix-511 silk	Elixirgen Scientific	NI511S
Minimum Essential Media (MEM) α, no nucleosides	ThermoFisher	12561056
KnockOut Serum Replacement (KSR)	ThermoFisher	10828010
Sodium Pyruvate (100 mM)	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 our Cell Dissociation Reagent (Solution D1), Catalog Number: CDR.

Workflow

* From Day 3, users may maintain differentiated cells in the maintenance medium best suited for their needs, though we recommend Quick-Endothelium™ Vascular Maintenance Medium, Catalog Number: VE-MM.

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 10 mM 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. This mixture, hereafter referred to as 10 mM SB431542, can be stored 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 E

1. Prepare Medium E using the reagents listed in the table below.
    - All reagents should be warmed at room temperature for 20-30 minutes.
2. Store Medium E for up to 2 weeks at 4°C.

Medium E 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

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

Medium VE Reagents	Volume
Medium E	25 ml
20 µg/ml VEGF-165	12.5 µl
10 mM SB431542 (1,000x)	25 µl

Experiment planning

Define the cell culture plate or dish format in advance and calculate the number of wells to be used for each format in advance. For example, you may use only a certain number of wells of a 96-well plate. The following section describes culture condition volumes per well as user needs may vary. When a 96-well plate is used, we recommend filling the edge wells of the plate with an aqueous medium instead of cells and culture medium. This will maintain humidity on the entire plate. Please refer to the table below for plate formats and corresponding surface area of each well used for calculating reagents in the following sections.

Plate format	6-well plate	24-well plate	96-well plate
Approximate cell growth surface area per well	9.5 cm^2	1.9 cm^2	0.32 cm^2

Day 0

5-6 hours

Plate Preparation

IMPORTANT! Cells can be plated in 6-well or 24-well plates depending on the desired format. Refer to the table at the bottom of this page for the recommended volumes per well.

1. Thaw iMatrix-511 silk on ice for 20-30 minutes (or at 4°C overnight one day before Day 0).
2. Take 2 ml ice-cold PBS into a tube and add 6.6 µl iMatrix-511 silk to it. Mix them well. Store the rest of iMatrix-511 silk at 4°C for its use at Day 8.
3. Select a proper cell culture size suited for your experiment. Add diluted iMatrix-511 silk according to the volumes listed in Table 1 or scale the volumes described in the protocol by the ratio between well sizes accordingly.

4. Incubate the 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 well and add a volume of PBS to it according to Table 1.
6. Incubate the plate at 37°C, 5% CO_2 until the cell suspension is ready for plating.

Table 1. Recommended volumes per well for different plate formats.

Reagents	Corresponding steps	Recommended volume per well	
		6-well plate	24-well plate
Diluted iMatrix-511 silk	3	2.0 ml	400 µl

Thawing Cells

1. Warm Medium E and Medium VE to room temperature for 20-30 minutes.
2. Take 4.5 ml Medium E into a 15 ml conical tube. Take out the vial of Quick-Endothelium™ Vascular (Frozen) from the liquid nitrogen storage tank.
3. Incubate the cryovial in a water bath set at 37°C (do not submerge the cap) until the most of the content is thawed but a small ice crystal remains (approximately 1-2 minutes).
4. Wipe the vial with a dry paper towel. Spray 70% ethanol to the vial and bring it inside a biosafety cabinet.
5. Take 0.5 ml Medium E from the conical tube using a P1000 pipettor and add into the cryovial dropwise at 1 drop / 1-2 seconds.
6. Using the same P1000 tip, gently pipet up and down the cell suspension once.
7. Transfer all cell suspension using the same P1000 tip to the 15 ml conical tube containing 4 ml Medium E. Mix them well by pipetting 3 times.
8. Centrifuge the cell suspension at 200 x g for 4 minutes.
9. While centrifugation is running, take 2 ml Medium VE into a tube and add 2 µl 10 mM ROCK inhibitor to it. Mix them well. This medium is referred to as Medium iVE.
10. Aspirate most of the supernatant from the centrifuged conical tube but leave a small volume of the supernatant (< 50 µl) to cover the pellet.
11. Simply tap the side of the conical tube 4-5 times to break the cell pellet.
12. Add 1 ml fresh Medium iVE to the conical tube with the cell pellet using a P1000 pipettor and pipet it up and down 2-3 times.

Plating Cells

1. Count the cells to determine the volume of cell suspension needed for the chosen number of wells and include a 10% buffer for cell number and volume. If the volume of the cell suspension needs to be adjusted, centrifuge the required volume of cell suspension at 200 x g for 4 minutes, remove the supernatant, and resuspend the pellet with Medium iVE to reach the multiplied volume of cell suspension with the number of wells.
2. Add cell suspension to the center of each well. Since each well already has Medium iVE, the total volume of the medium in each well is indicated in the table below.
3. Incubate at 37°C, 5% CO_2 overnight.

Table 2. Recommended cell plating density

	Recommended amounts	
	6-well plate	24-well plate
Required total volume of cell suspension • (Volume of cell suspension/well) + 10% buffer	550 µl	110 µl
Volume of cell suspension distributed/well	500 µl	100 µl
Total volume/well • Medium iVE + cell suspension	1 ml	300 µl

Day 1+ (or until the culture reaches nearly 80-90% confluency)

< 1 hour

Maintenance

1. Warm Medium VE at room temperature for 20-30 minutes.
2. Pipet out the old medium from each well and add Medium VE to each well along its wall according to the table below.

Required volume per well	6-well plate	24-well plate
Medium VE	4 ml	800 µl

3. Incubate the culture at 37°C, 5% CO_2 for 2 days.
4. Repeat steps 1-3 every 2 days until the culture reaches close to 90% confluency. Do not let the culture overgrow. Cells can be passaged 2 more times.

Day 3+

< 1 hour

Assay or Continuous Maturation

- Differentiated vascular endothelial cells can be observed on Day 1. For more mature cells, we recommend culturing cells until Day 3. From Day 3, users may maintain differentiated cells in the medium best suited for their needs, though we recommend Quick-Endothelium™ Vascular Maintenance Medium, Catalog Number: VE-MM.
- Differentiation into vascular endothelial cells after using Quick-Endothelium™ Vascular - Human iPSC-derived cells can be confirmed with the endothelial cell markers CD31, CLDN5, and VWF.
- Cells can be cultured for up to 3 passages post-thaw.

Appendix

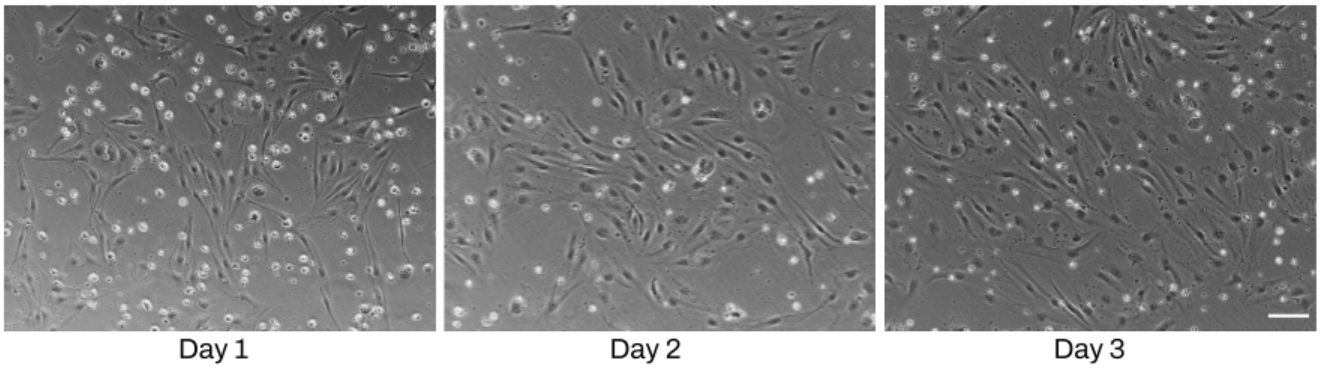

Figure 1. Representative phase contrast images of Quick-Endothelium™ Vascular - Human iPSC-derived vascular endothelial cell cultures on post-thaw days 1, 2, and 3 post-thaw (scale bar = 100 µm).

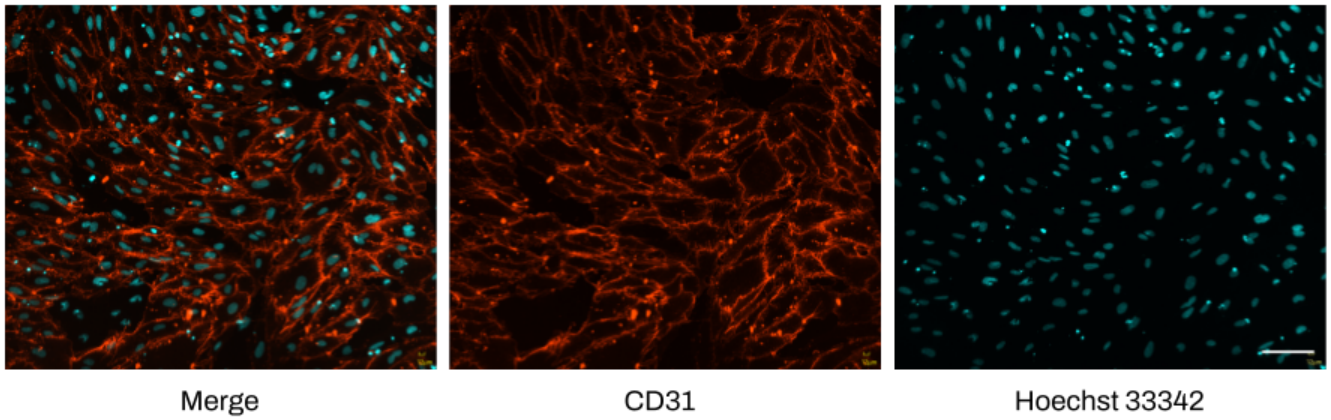

Figure 2. Immunofluorescent staining of Quick-Endothelium™ Vascular - Human iPSC-derived vascular endothelial- mRNA- CW Kit cell cultures shows typical vascular endothelial cell morphology and expression of CD31 on day 3 post-thaw (scale bar = 100 µm). Staining conditions:Purified anti-human CD31 antibody (Biolegend, Catalog number: 303102, 1:50 dilution) was used in combination with a secondary antibody (Invitrogen, Catalog number: A11032 Goat anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594, 1:500 dilution). Nuclei were counterstained with Hoechst 33342.