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Clonetics™ Endothelial Cell System

Technical Information & Instructions

Contents:

I. Introduction

Clonetics™ endothelial cell systems offer both normal and diseased human endothelial cells and optimized media for their growth. Each system can quickly generate endothelial cultures for a variety of experimental applications depending on the cell type including cardiovascular pharmaceutical development, vascular pathology, atherosclerosis, cardiovascular research, circulatory physiology, wound healing, angiogenesis, lymphangiogenesis, tumorigenesis, oncology, immunology, the study of bladder function, drug development and basic research. Clonetics™ Endothelial Cell Systems are convenient and easy to use, allowing the researcher to focus on results. Clonetics™ cells, medium and reagents are quality tested together and guaranteed to give optimum performance as a complete cell system.

For answers to frequently asked questions regarding these products, please visit our FAQ Database:

www.lonza.com/faq

For citations citing the use of these products, please visit our Citations Database:

www.lonza.com/citations

II. General Cell Information: Normal Cells

Cat. No.	Description	Recommended Growth Media	Cryopreserved Passage Number	Proliferating Passage Number*	Seeding Density Upon Thaw**	Time to Subculture
CC-2535	Aortic Endothelial (HAEC)	EGM™-2 BulletKit™ Medium	Passage 3	Passage 4	5,000 viable cells/cm^2	5-9 days
CC-2585	Coronary Artery Endothelial (HCAEC)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4	5,000 viable cells/cm^2	5-9 days
CC-2545	Iliac Artery Endothelial (HIAEC)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4	5,000 viable cells/cm^2	5-9 days
CC-2530	Pulmonary Artery Endothelial (HPAEC)	EGM™-2 BulletKit™ Medium	Passage 3	Passage 4	5,000 viable cells/cm^2	5-9 days
CC-2519	Umbilical Vein Endothelial, Pooled Donors (HUVEC)	EGM™ BulletKit™ Medium	Passage 1	Passage 2	2,500 viable cells/cm^2	5-9 days
C2519A	Umbilical Vein Endothelial, Pooled Donors (HUVEC)	EGM™-2 BulletKit™ Medium	Passage 1	Passage 2	2,500 viable cells/cm^2	5-9 days
C2519AS	Umbilical Vein Endothelial, Pooled Donors, Prescreened (HUVEC)	EGM™-2 BulletKit™ Medium	Passage 1	Passage 2	2,500 viable cells/cm^2	5-9 days
00191027	Umbilical Vein Endothelial, Pooled Donors (HUVEC-XL)	EGM™-2 BulletKit™ Medium	Passage 3	N/A	2,500 viable cells/cm^2	5-9 days
CC-2517	Umbilical Vein Endothelial, Single Donor (HUVEC)	EGM™ BulletKit™ Medium	Passage 1	Passage 2	2,500 viable cells/cm^2	5-9 days
C2517A	Umbilical Vein Endothelial, Single Donor (HUVEC)	EGM™-2 BulletKit™ Medium	Passage 1	Passage 2	2,500 viable cells/cm^2	5-9 days
C2517AS	Umbilical Vein Endothelial, Single Donor, Prescreened (HUVEC)	EGM™-2 BulletKit™ Medium	Passage 1	Passage 2	2,500 viable cells/cm^2	5-9 days
CC-2935	Umbilical Vein Endothelial, Single Donor (HUVEC)	EGM™-PLUS BulletKit™ Medium	Passage 1	Passage 2	2,500 viable cells/cm^2	5-9 days
CC-7016	Bladder Microvascular Endothelial (HMVEC-Bd)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-7030	Cardiac Microvascular Endothelial (HMVEC-C)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2811	Dermal Blood Microvascular Endothelial, Adult (HMVEC-DBlAd)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2543	Dermal Microvascular Endothelial, Adult (HMVEC-DAd)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2516	Dermal Microvascular Endothelial, Neonatal, Pooled Donors (HMVEC-DNeo)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2505	Dermal Microvascular Endothelial, Neonatal, Single Donor (HMVEC-DNeo)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2813	Dermal Blood Microvascular Endothelial, Neonatal (HMVEC-DBlNeo)	EGM™-2MV BulletKit™ Medium	Passage 4	Passage 5 or 6	5,000 viable cells/cm^2	5-9 days
CC-2810	Dermal Lymphatic Microvascular Endothelial, Adult (HMVEC-DLyAd)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2812	Dermal Lymphatic Microvascular Endothelial, Neonatal (HMVEC-DLyNeo)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2527	Lung Microvascular Endothelial (HMVEC-L)	EGM™-2MV BulletKit™ Medium	Passage 3 or 4	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2814	Lung Lymphatic Microvascular Endothelial (HMVEC-LLy)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2564	Myometrial Uterine Microvascular Endothelial (UtMVEC-Myo)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days

*Proliferating cultures are generated using Lonza's cryopreserved cell stock. Proliferating cultures are delivered Tuesday-Thursday the week after initial plating and confluence at the time of shipment varies by cell type. Proliferating cultures are available in a variety of culture vessels including flasks and well plates. For more information regarding proliferating cultures, including catalog numbers, please contact Lonza Scientific Support.

**Please note that alternative seeding densities may be required for subculture and/or differentiation.

III. General Cell Information: Diseased Cells

Cat. No.	Description	Recommended Growth Media	Cryopreserved Passage Number	Proliferating Passage Number*	Seeding Density Upon Thaw**	Time to Subculture
CC-2919	Diseased Aortic Endothelial, Diabetic Type I (D-HAEC)	EGM™-2 BulletKit™ Medium	Passage 3	Passage 4	5,000 viable cells/cm^2	5-9 days
CC-2920	Diseased Aortic Endothelial, Diabetic Type II (D-HAEC)	EGM™-2 BulletKit™ Medium	Passage 3	Passage 4	5,000 viable cells/cm^2	5-9 days
CC-2921	Diseased Coronary Artery Endothelial, Diabetic Type I (D-HCAEC)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2922	Diseased Coronary Artery Endothelial, Diabetic Type II (D-HCAEC)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2923	Diseased Pulmonary Artery Endothelial, Diabetic Type I (D-HPAEC)	EGM™-2 BulletKit™ Medium	Passage 3	Passage 4	5,000 viable cells/cm^2	5-9 days
CC-2924	Diseased Pulmonary Artery Endothelial, Diabetic Type II (D-HPAEC)	EGM™-2 BulletKit™ Medium	Passage 3	Passage 4	5,000 viable cells/cm^2	5-9 days
CC-2927	Diseased Cardiac Microvascular Endothelial, Diabetic Type I (D-HMVEC-C)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2928	Diseased Cardiac Microvascular Endothelial, Diabetic Type II (D-HMVEC-C)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2929	Diseased Dermal Microvascular Endothelial, Adult, Diabetic Type I (D-HMVEC-DAd)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days
CC-2930	Diseased Dermal Microvascular Endothelial, Adult, Diabetic Type II (D-HMVEC-DAd)	EGM™-2MV BulletKit™ Medium	Passage 3	Passage 4 or 5	5,000 viable cells/cm^2	5-9 days

*Proliferating cultures are generated using Lonza's cryopreserved cell stock. Proliferating cultures are delivered Tuesday-Thursday the week after initial plating and confluence at the time of shipment varies by cell type. Proliferating cultures are available in a variety of culture vessels including flasks and well plates. For more information regarding proliferating cultures, including catalog numbers, please contact Lonza Scientific Support.

**Please note that alternative seeding densities may be required for subculture and/or differentiation.

IV. Quality Control: Normal Cells

Cat. No.	Description	Cells/Vial	Viability	Maximum Population Doublings	Doubling Time	Properties
CC-2535	Aortic Endothelial (HAEC)	≥500,000 cells	≥70%	≥15	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2585	Coronary Artery Endothelial (HCAEC)	≥500,000 cells	≥70%	≥15	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2545	Iliac Artery Endothelial (HIAEC)	≥500,000 cells	≥70%	≥10	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2530	Pulmonary Artery Endothelial (HPAEC)	≥500,000 cells	≥70%	≥15	11.5-32.5 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2519	Umbilical Vein Endothelial, Pooled Donors (HUVEC)	≥500,000 cells	≥70%	≥15	15-48 hrs	CD31$^+$/CD105$^+$
C2519A	Umbilical Vein Endothelial, Pooled Donors (HUVEC)	≥500,000 cells	≥70%	≥15	12-48 hrs	CD31$^+$/CD105$^+$
C2519AS	Umbilical Vein Endothelial, Pooled Donors, Prescreened (HUVEC)	≥500,000 cells	≥70%	≥15	12-48 hrs	CD31$^+$/CD105$^+$ Axl$^+$; eNOS$^+$; Tie-2$^+$; VEGFr2$^+$
00191027	Umbilical Vein Endothelial, Pooled Donors (HUVEC-XL)	≥10,000,000 cells	≥70%	≥5	12-48 hrs	CD31$^+$/CD105$^+$; Alpha Actin$^-$; Factor VIII$^+$; Acetylated LDL Uptake$^+$
CC-2517	Umbilical Vein Endothelial, Single Donor (HUVEC)	≥500,000 cells	≥70%	≥15	15-48 hrs	CD31$^+$/CD105$^+$
C2517A	Umbilical Vein Endothelial, Single Donor (HUVEC)	≥500,000 cells	≥70%	≥15	12-48 hrs	CD31$^+$/CD105$^+$
C2517AS	Umbilical Vein Endothelial, Single Donor, Prescreened (HUVEC)	≥500,000 cells	≥70%	≥15	12-48 hrs	CD31$^+$/CD105$^+$ Axl$^+$; eNOS$^+$; Tie-2$^+$; VEGFr2$^+$
CC-2935	Umbilical Vein Endothelial, Single Donor (HUVEC)	≥500,000 cells	≥70%	≥15	15-48 hrs	CD31$^+$/CD105$^+$
CC-7016	Bladder Microvascular Endothelial (HMVEC-Bd)	≥500,000 cells	≥70%	≥10	12-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-7030	Cardiac Microvascular Endothelial (HMVEC-C)	≥500,000 cells	≥70%	≥10	12-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2811	Dermal Blood Microvascular Endothelial, Adult (HMVEC-DBlAd)	≥500,000 cells	≥70%	≥12	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2543	Dermal Microvascular Endothelial, Adult (HMVEC-DAd)	≥500,000 cells	≥70%	≥15	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2516	Dermal Microvascular Endothelial, Neonatal, Pooled Donors (HMVEC-DNeo)	≥500,000 cells	≥70%	≥15	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2505	Dermal Microvascular Endothelial, Neonatal, Single Donor (HMVEC-DNeo)	≥500,000 cells	≥70%	≥15	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2813	Dermal Blood Microvascular Endothelial, Neonatal (HMVEC-DBlNeo)	≥500,000 cells	≥70%	≥12	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$; CD31$^+$; Podoplanin$^-$
CC-2810	Dermal Lymphatic Microvascular Endothelial, Adult (HMVEC-DLyAd)	≥500,000 cells	≥70%	≥12	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$; CD31$^+$; Podoplanin$^+$
CC-2812	Dermal Lymphatic Microvascular Endothelial, Neonatal (HMVEC-DLyNeo)	≥500,000 cells	≥70%	≥12	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$; CD31$^+$; Podoplanin$^+$
CC-2527	Lung Microvascular Endothelial (HMVEC-L)	≥500,000 cells	≥70%	≥15	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$; PECAM$^+$
CC-2814	Lung Lymphatic Microvascular Endothelial (HMVEC-LLy)	≥500,000 cells	≥70%	≥12	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$; CD31$^+$; Podoplanin$^+$
CC-2564	Myometrial Uterine Microvascular Endothelial (UtMVEC-Myo)	≥500,000 cells	≥70%	≥15	15-48 hrs	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B, Hepatitis-C, bacteria, yeast and fungi. Cell viability, morphology, cell number, and proliferative capacity are measured after recovery from cryopreservation. Clonetics™ Media are formulated for optimal growth of specific types of human cells. COAs for all cell products are available upon request. Please see Section XVIII (Product Warranty, Page 13) for more information on Quality Control claims and guarantees.

V. Quality Control: Diseased Cells

Cat. No.	Description	Cells/Vial	Viability	Maximum Population Doublings	Doubling Time	Properties
CC-2919	Diseased Aortic Endothelial, Diabetic Type I (D-HAEC)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2920	Diseased Aortic Endothelial, Diabetic Type II (D-HAEC)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2921	Diseased Coronary Artery Endothelial, Diabetic Type I (D-HCAEC)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2922	Diseased Coronary Artery Endothelial, Diabetic Type II (D-HCAEC)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2923	Diseased Pulmonary Artery Endothelial, Diabetic Type I (D-HPAEC)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2924	Diseased Pulmonary Artery Endothelial, Diabetic Type II (D-HPAEC)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2927	Diseased Cardiac Microvascular Endothelial, Diabetic Type I (D-HMVEC-C)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2928	Diseased Cardiac Microvascular Endothelial, Diabetic Type II (D-HMVEC-C)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2929	Diseased Dermal Microvascular Endothelial, Adult, Diabetic Type I (D-HMVEC-DAd)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$
CC-2930	Diseased Dermal Microvascular Endothelial, Adult, Diabetic Type II (D-HMVEC-DAd)	≥500,000 cells	≥70%	FIO*	FIO*	Alpha Actin$^-$; Factor VIII$^+$ Acetylated LDL Uptake$^+$

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B, Hepatitis-C, bacteria, yeast and fungi. Cell viability, morphology, cell number, and proliferative capacity are measured after recovery from cryopreservation. Clonetics™ Media are formulated for optimal growth of specific types of human cells. COAs for all cell products are available upon request. Please see Section XVIII (Product Warranty, Page 13) for more information on Quality Control claims and guarantees.

*For Information Only: Cells must reach ≥90% confluence in the first passage from cryopreservation and are continued in culture until Day 7±2 in the second passage from cryopreservation. The total number of population doublings obtained and the doubling time during this period are reported for information only on the Certificate of Analysis.

VI. Quality Control: Media & Reagents

Basal Media

Description:	EBM™ Basal Medium/ EBM™ Phenol Red Free Basal Medium	EBM™-2 Basal Medium	EBM™ PLUS Basal Medium
Catalog No.	CC-3121 / CC-3129	CC-3156 / 00190860	CC-5036
Test	**Specification**		
Sterility	Negative	Negative	Negative
pH	7.5 – 8.0	7.6 – 8.0	7.4 – 8.0
Osmolality (mOsm/kg H_2O)	260-290	260-290	260-290
Endotoxin	FIO*	FIO*	FIO*

*For Information Only

Large Vessel SingleQuots™ Kits

Description:	EGM™ SingleQuots™ Kit	EGM™-PLUS SingleQuots™ Kit	EGM™-2 SingleQuots™ Kit
Catalog No.	CC-4133	CC-4542	CC-4176
Test	**Specification**		
Sterility	Negative	Negative	Negative
Performance Test	Pass	Pass	Pass

Microvascular SingleQuots™ Kits

Description:	EGM™-2MV SingleQuots™ Kit
Catalog No.	CC-4147
Test	**Specification**
Sterility	Negative
Performance Test	Pass

Subculture Reagents

Description:	Trypsin/ EDTA	Trypsin Neutralizing Solution (TNS)	HEPES Buffered Saline Solution
Catalog No.	CC-5012	CC-5002	CC-5022 / CC-5024
Test	**Specification**		
Sterility	Negative	Negative	Negative
Performance Test	Pass	N/A	Pass
pH	FIO*	N/A	FIO* (Target: 7.15 – 7.55)
Osmolality (mOsm/kg H_2O)	FIO*	N/A	FIO* (Target: 287 - 317)
Endotoxin (EU/ml)	N/A	N/A	FIO* (Target: ≤0.05)
*For Information Only			

VII. Cell Growth System Components
(Sold Separately)

- One human endothelial cell product – (cryopreserved or proliferating)
- One Endothelial Cell Media BulletKit™ Medium - 500 ml for the proliferation of human, large vessel endothelial cells in a medium containing no exogenous Vascular Endothelial Growth Factor (VEGF).

  Clonetics™ EGM BulletKit™ (Lonza Catalog No. CC-3124) contains 500 ml of Endothelial Basal Medium-PLUS (EBM™ Medium) and the following growth supplements: Bovine Brain Extract (BBE), 2.0 ml; Ascorbic Acid, 0.5 ml; Hydrocortisone, 0.5 ml; human Epidermal Growth Factor (hEGF), 0.5 ml; Fetal Bovine Serum (FBS), 10.0 ml; Gentamicin/Amphotericin-B (GA), 0.5 ml

  Or

  One Endothelial Cell Media BulletKit™ Medium - 500 ml for the enhanced proliferation of human, large vessel endothelial cells in a medium containing no exogenous Vascular Endothelial Growth Factor (VEGF) and no phenol red.

  Clonetics™ EGM™-PLUS BulletKit™ (Lonza Catalog No. CC-5035) contains 500 ml of Endothelial Basal Medium-PLUS (EBM™-PLUS Medium) and the following growth supplements: Endothelial Growth Supplement (EnGS), 1.0 ml; L-Glutamine, 25.0 ml; Ascorbic Acid, 0.5 ml; Hydrocortisone Hemisuccinate, 0.5 ml; human Epidermal Growth Factor (hEGF), 0.5 ml; Heparin (0.5 ml); Fetal Bovine Serum (FBS), 10.0 ml; Gentamicin/Amphotericin-B (GA), 0.5 ml

  Or

  One Endothelial Cell Media BulletKit™ Medium - 500 ml for the rapid proliferation of human, large vessel endothelial cells in a medium containing Vascular Endothelial Growth Factor (VEGF).

  Clonetics™ EGM™-2 BulletKit™ (Lonza Catalog No. CC-3162) contains 500 ml of Endothelial Basal Medium-2 (EBM™-2 Medium) and the following growth supplements: human Epidermal Growth Factor (hEGF), 0.5 ml; Vascular Endothelial Growth Factor (VEGF), 0.5 ml; R3-Insulin-like Growth Factor-1 (R3-IGF-1), 0.5 ml; Ascorbic Acid, 0.5 ml; Hydrocortisone, 0.2 ml; human Fibroblast Growth Factor-Beta (hFGF-β), 2.0 ml; Heparin (0.5 ml); Fetal Bovine Serum (FBS), 10.0 ml; Gentamicin/Amphotericin-B (GA), 0.5 ml

  Or

  One Endothelial Cell Media BulletKit™ Medium - 500 ml for the rapid proliferation of human, microvascular endothelial cells in a medium containing Vascular Endothelial Growth Factor (VEGF).

  Clonetics™ EGM™-2MV BulletKit™ (Lonza Catalog No. CC-3202) contains 500 ml of Endothelial Basal Medium-2 (EBM™-2 Medium) and the following growth supplements: human Epidermal Growth Factor (hEGF), 0.5 ml; Vascular Endothelial Growth Factor (VEGF), 0.5 ml; R3-Insulin-like Growth Factor-1 (R3-IGF-1), 0.5 ml; Ascorbic Acid, 0.5 ml; Hydrocortisone, 0.2 ml; human Fibroblast Growth Factor-Beta (hFGF-β), 2.0 ml; Fetal Bovine Serum (FBS), 25.0 ml; Gentamicin/Amphotericin-B (GA), 0.5 ml
- One ReagentPack™ Subculture Reagents (Lonza Catalog No. CC-5034), containing:

Trypsin/EDTA	100 ml
Trypsin Neutralizing Solution	100 ml
HEPES Buffered Saline Solution	100 ml

NOTE: Additional components are necessary for the cryopreservation of these cells. Please see the corresponding selection below for more information.

VIII. Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells: Remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact Customer Service.
3. For proliferating cells: Swab down the flask of proliferating cells with 70% ethanol or isopropanol, then place the flask in 37℃, 5% CO_2, humidified incubator and allow to equilibrate for three to four hours. After cells have equilibrated, remove shipping medium from the flask and replace with fresh medium.
4. BulletKit™ Medium instructions: store basal medium protected from light at 2°-8℃ and SingleQuots™ Kit at ≤-20℃ in a freezer that is not self-defrosting. Once thawed, SingleQuots™ Kit should be stored at 2°-8℃ and added to basal medium within 72 hours. After SingleQuots™ Kit is added to basal medium, store protected from light at 2°-8℃ and use within 1 month. Do not re-freeze.
5. ReagentPack™ Subculture Reagents are sterile-filtered and then stored at –20℃ until shipment. Subculture reagents may thaw during transport. They may be refrozen once. If you plan to use within 3 days, store at 4℃. Trypsin/EDTA Solution has a limited shelf life or activation at 4℃. If, upon arrival, Trypsin/EDTA is thawed, immediately aliquot and refreeze at –20℃. We recommend that the HEPES-BSS and the Trypsin Neutralizing Solution be stored at 4℃ for no more than one month.

NOTE: To keep Trypsin/EDTA fresh and active after thawing, you may aliquot it into sterile centrifuge tubes and re-freeze at –20°C.

Lonza guarantees the performance of Clonetics™ cells only if appropriate Clonetics™ media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

IX. Preparation of Culture Media

1. Decontaminate external surfaces of all vials, including the medium bottle, with ethanol or isopropanol.
2. To formulate Endothelial Growth Medium (EGM™ Medium), transfer the contents of the EGM™ SingleQuots™ Kit (Lonza Catalog No. CC-4133 containing Bovine Brain Extract [BBE]; Ascorbic Acid, Hydrocortisone, Epidermal Growth Factor [hEGF], Fetal Bovine Serum [FBS] and Gentamicin/Amphotericin-B [GA]) to EBM™ Basal Medium with a pipette, and rinse each vial with medium.
3. To formulate Endothelial Growth Medium-PLUS (EGM™-PLUS Medium), transfer the contents of the EGM™-PLUS SingleQuots™ Kit (Lonza Catalog No. CC-4542 containing Endothelial Growth Supplement [EnGS], L-Glutamine, Ascorbic Acid, Hydrocortisone Hemisuccinate, human Epidermal Growth Factor [hEGF], Heparin, Fetal Bovine Serum [FBS] and Gentamicin/Amphotericin-B [GA]) to EBM™-PLUS Basal Medium with a pipette, and rinse each vial with medium.
4. To formulate Endothelial Growth Medium-2 (EGM™-2 Medium), transfer the contents of the EGM™-2 SingleQuots™ Kit (Lonza Catalog No. CC-4176 containing human Epidermal Growth Factor [hEGF], Vascular Endothelial Growth Factor [VEGF], R3-Insulin-like Growth Factor-1 [R3-IGF-1], Ascorbic Acid, Hydrocortisone, human Fibroblast Growth Factor-Beta [hFGF-β], Heparin, Fetal Bovine Serum [FBS], and Gentamicin/Amphotericin-B [GA]) to EBM™-2 Basal Medium with a pipette, and rinse each vial with medium.
5. To formulate Microvascular Endothelial Growth Medium-2 (EGM™-2MV Medium), transfer the contents of the EGM™-2MV SingleQuots™ Kit (Lonza Catalog No. CC-4147 containing human Epidermal Growth Factor [hEGF], Vascular Endothelial Growth Factor [VEGF], R3-Insulin-like Growth Factor-1 [R3-IGF-1], Ascorbic Acid, Hydrocortisone, human Fibroblast Growth Factor-Beta [hFGF-β], Fetal Bovine Serum [FBS], and Gentamicin/Amphotericin-B [GA]) to EBM™-2 Basal Medium with a pipette, and rinse each vial with medium.

6. When preparing these BulletKit™ Media, it may not be possible to recover the entire volume listed for each vial. Small losses (up to 10%) should not affect the cell growth characteristics of the supplemented medium.
7. Transfer the label provided with each kit to the basal medium bottle(s) being supplemented (avoid covering the basal medium lot # and expiration date). Use it to record the date and amount of each supplement added. After SingleQuots™ Kit is added to basal medium, store at 2°-8°C and use within 1 month. Do not freeze medium.

NOTE: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be re-filtered with a 0.2 µm filter to assure sterility. Routine re-filtration is not recommended.

X. Thawing of Cells / Initiation of Culture Process

NOTE: For proliferation of these cells, cells must be cultured at 37°C±1°C, 5% CO_2, 90%±2% humidity.

1. When initially plating endothelial cells from cryopreservation, the recommended seeding density is provided in the table below:

Cell Type	Recommended Seeding Density from Cryopreservation	Minimum Number of Flasks to Plate*
HUVEC	2,500 viable cells/cm^2	≥5 x T-25 flasks OR ≥1 x T-75 flask
HUVEC-XL	2,500 viable cells/cm^2	≥12 x T-225 flasks OR
Non-HUVEC Endothelial	5,000 viable cells/cm^2	≥2 x T-25 flasks OR ≥1 x T-75 flask

*Calculations based on the minimum guaranteed cell count and viability. Please consult the product COA for exact cell count and viability when determining the appropriate number of flasks to plate. Alternative plate sizes/formats may be used so long as the appropriate seeding density is achieved.

2. To set up culture vessels, calculate the number of vessels needed based on the recommended seeding density as well as the surface area of the vessels being used.
3. Add the appropriate amount of medium to the vessels (1 ml/5 cm^2) and allow the vessels to equilibrate in a 37°C±1°C, 5% CO_2, 90%±2% humidity incubator for at least 30 minutes.
4. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure and then retighten. Quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial. Watch your cryovial closely; when the last sliver of ice melts, remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.

NOTE: Centrifugation should **not** be performed to remove cells from cryoprotectant cocktail. This action is more damaging than the effects of DMSO residue in the culture.

5. Carefully mix the cell suspension using a micropipette. Dispense cells into the culture vessels set up in previous steps. Gently rock the culture vessel to evenly distribute the cells and return to the 37°C±1°C, 5% CO_2, 90%±2% humidity incubator.

NOTE: Endothelial cells tend to more strongly adhere to the cryovial than other cell types. Additional and/or more forceful trituration may be necessary to remove all cells.

6. Change the growth medium 16 to 24 hours after seeding.

XI. Maintenance

1. Change the growth medium 16 to 24 hours after seeding and every other day (every 48 hours) thereafter.
2. When cell confluence is 25-45%, increase the media volume to 1.5 ml/5 cm^2.
3. When cell confluence is greater than 45%, increase the media volume to 2 ml/5 cm^2.
4. Warm an appropriate amount of medium to 37°C in a sterile container. Remove the medium and replace it with the warmed, fresh medium and return the flask to the incubator.
5. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.

XII. Subculturing

NOTE: Lonza warrants its Clonetics™ Cells only if Lonza Subculturing Reagents are used. The recommended subculturing reagents for these cells are Trypsin/EDTA (CC-5012), Trypsin Neutralizing Solution (CC-5002), and HEPES Buffered Saline Solution (CC-5022). These reagents can be purchased individually or together as part of the Reagent Pack™ Subculture Reagents (CC-5034).

The following instructions are for a 25 cm^2 flask. Adjust all volumes accordingly for other size flasks.

1. Subculture the cells when they are 70%-85% confluent.
2. For each 25 cm^2 of cells to be subcultured:
   a. Thaw 2 ml of Trypsin/EDTA and allow to come to room temperature.
   b. Allow 7-10 ml of HEPES Buffered Saline Solution (HEPES-BSS) to come to room temperature.
   c. Allow 5 ml of Trypsin Neutralizing Solution (TNS) to come to room temperature.
   d. Remove growth medium from 4°C storage and allow warming to room temperature.
   e. Prepare new culture vessels.
3. Subculture one flask at a time. All flasks following the first flask will be subcultured following an optimization of this protocol (explained later in this procedure), based on calculated cell count, cell viability, and seeding density.

NOTE: The following steps must be performed in a sterile field.

4. Aspirate the medium from one culture vessel.
5. Rinse the cells with 5 ml of room temperature HEPES-BSS. DO NOT forget this step. The medium contains complex proteins and calcium that neutralize the trypsin.
6. Aspirate the HEPES-BSS from the flask.
7. Cover the cells with 2 ml of Trypsin/EDTA solution.
8. Place the culture vessels into a 37°C humidified incubator for 3-5 minutes. Periodically examine the cell layer microscopically and check for cell detachment.
9. Allow the trypsinization to continue until approximately 90% of the cells are rounded up.
10. At this point, tap the flask against the palm of your hand to release the majority of cells from the culture surface. If only a few cells detach, you may not have let them trypsinize long enough. Wait 30 seconds and tap again. If cells still do not detach, wait and tap every 30 seconds thereafter. This entire process should take no more than 5 minutes.

NOTE: If the majority of cells does not detach within 5 minutes, the trypsin is either not warm enough or not active enough to release the cells. Harvest the culture vessel as described below, and either re-trypsinize with fresh, warm Trypsin/EDTA solution or rinse with Trypsin Neutralizing Solution and then add fresh, warm medium to the culture vessel. Return to an incubator until fresh trypsinization reagents are available.

11. After cells are released, neutralize the trypsin in the flask with 5 ml of Trypsin Neutralizing Solution at room temperature.
12. Quickly transfer the detached cells to a sterile 15 ml centrifuge tube.
13. Rinse the flask with a final 2 ml of HEPES-BSS to collect residual cells, and add this rinse to the centrifuge tube.
14. Examine the harvested flask under the microscope to make sure the harvest was successful by looking at the number of cells left behind. This should be less than 5%.
15. Centrifuge the harvested cells at 200 x g for five minutes to pellet the cells.
    - Aspirate most of the supernatant, except for 100-200 µl
    - Flick the cryovial with your finger to loosen the pellet
16. Dilute the cells to a final volume of 2 to 3 ml of growth medium and note the total volume of the diluted cell suspension.
17. Determine cell count and viability using a hemacytometer and Trypan Blue. Make a note of your cell yield for later use.
18. If necessary, dilute the suspension with growth medium to achieve the desired “cells/ml” and re-count the cells.
19. Use the following equation to determine the total number of viable cells.

$$\text{Total \# of Viable Cells} = \frac{\text{Total cell count} \times \text{percent viability}}{100}$$

20. The number of flasks needed depends upon cell yield, cell type, seeding density, and application. The recommended seeding density when subculturing endothelial cells for further proliferation or angiogenesis is provided in the table below:

Cell Type/Application	Recommended Seeding Density after Subculture
HUVEC	2,500 viable cells/cm^2
HMVEC-DNeo	2,000 viable cells/cm^2
HMVEC-LLy	2,500 - 5,000 viable cells/cm^2
All Other Endothelial	5,000 viable cells/cm^2
Angiogenesis	65,000 – 80,000 viable cells/cm^2

Determine the total number of flasks to inoculate by using the following equation.

$$\text{Total \# of Flasks to innoculate} = \frac{\text{Total \# of viable cells}}{\text{Growth area} \times \text{Rec. Seeding Density}}$$

21. Use the following equation to calculate the volume of cell suspension to seed into your flasks.

$$\text{Seeding Volume} = \frac{\text{Total volume of diluted cell suspension}}{\text{\# of flasks as determined in step 18}}$$

22. Prepare flasks by labeling each flask with the passage number, cell type, and date.
23. If seeding into flasks or well plates for angiogenesis, coat each vessel with Matrigel® Basement Membrane Matrix as described in Section XV (Angiogenesis: Plate Coating, Page 11).

NOTE: Plate coating is not necessary for standard growth and proliferation of Lonza endothelial cells.

24. Carefully transfer growth medium to new culture vessels by adding 1 ml growth medium for every 5 cm^2 surface area of the flask (1 ml/5 cm^2) for further culturing of the cells or for differentiation of the cells.
25. After mixing the diluted cells with a 5 ml pipet to ensure a uniform suspension, dispense the calculated volume into the prepared subculture flasks.
26. If not using vented caps, loosen caps of flasks. Place the new culture vessels into a 37℃±1℃, 5% CO_2, 90%±2% humidity incubator.

XIII. Cryopreservation

NOTE: Cryopreservation may compromise cell quality and performance. **Lonza CANNOT guarantee performance of Clonetics™ & Poietics™ Cells that have been cryopreserved outside of Lonza.** To avoid loss of cells and forfeiture of your warranty, we recommend keeping cells in continuous culture without cryopreservation.

Cryopreservation Media

Description	Base Media	DMSO	FBS
HAEC	80% EGM™-2	10% DMSO	10% FBS
HCAEC	80% EGM™-2MV	10% DMSO	10% FBS
HIAEC	80% EGM™-2MV	10% DMSO	10% FBS
HPAEC	80% EGM™-2	10% DMSO	10% FBS
HUVEC (cultured in EGM™)	80% EGM™	10% DMSO	10% FBS
HUVEC (cultured in EGM™-PLUS)	80% EGM™-PLUS	10% DMSO	10% FBS
HUVEC (cultured in EGM™-2)	80% EGM™-2	10% DMSO	10% FBS
HMVEC-Bd	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-C	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-DBlAd	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-DAd	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-DNeo	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-DNeo	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-DBlNeo	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-DLyAd	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-DLyNeo	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-L	80% EGM™-2MV	10% DMSO	10% FBS
HMVEC-LLy	80% EGM™-2MV	10% DMSO	10% FBS
UtMVEC-Myo	80% EGM™-2MV	10% DMSO	10% FBS
D-HAEC (Type I and II)	80% EGM™-2	10% DMSO	10% FBS
D-HCAEC (Type I and II)	80% EGM™-2MV	10% DMSO	10% FBS
D-HPAEC (Type I and II)	80% EGM™-2	10% DMSO	10% FBS
D-HMVEC-C (Type I and II)	80% EGM™-2MV	10% DMSO	10% FBS
D-HMVEC-Dad (Type I and II)	80% EGM™-2MV	10% DMSO	10% FBS

1. Prepare cryopreservation media according to the chart listed above and chill to 4℃.
2. Prepare freezing vials or ampoules by labeling each with the passage number, cell type and date.
3. Sterile filter cryopreservation media using a 0.2 micron filter
4. Harvest and centrifuge cells according to Steps 1 to 15 of Section XII (Subculturing, Page 8).

5. Resuspend cells in cold cryopreservation media at 500,000 to 2,000,000 cells per ml.

NOTE: Work Quickly! Once exposed to the DMSO, cells become very fragile.

6. Pipet aliquots (1 ml each) into freezing vials or ampoules and seal.
7. Insulate aliquots with Styrofoam or propanol freezing canister.
8. Store cells at -80℃ overnight.
9. Within 12 to 24 hours, place cells in liquid nitrogen (-200℃) for long-term storage. Cells will be compromised by storage in -80℃.

XIV. Angiogenesis: Introduction

NOTE: This procedure is a recommendation only. Lonza endothelial cells are not quality control tested for angiogenesis and angiogenesis is not guaranteed under the cell warranty.

Angiogenesis is a multi-step process involving the generation new blood vessels from pre-existing vasculature and is mediated primarily by endothelial cells. It involves multiple steps like basement membrane disruption, endothelial cell migration, invasion, proliferation and differentiation into capillaries. *In vitro,* the process has various phases such as cell migration and alignment, followed by the development of capillary tubes, sprouting of new capillaries, and finally the formation of the cellular networks that result in a timed manner. Thus, varying the time point of measurement may give some information regarding the mechanism of action of an anti-angiogenic agent and the steps in the pathway it affects.

A number of inhibitors have been used in the literature as positive inhibitor controls for this assay. One of these inhibitors is Suramin. Suramin is a specific and competitive inhibitor of G-protein-coupled receptor (GPCR) activity and affects multiple outputs of tubule formation for example, number of junctions, number of tubules and the total tubule length (1).

Once tube formation is complete, it can be observed using an inverted microscope either in bright field, or after staining with a live cell staining dye like Calcein AM. Staining with Calcein AM enables better visualization of the tubules. Image acquisition can then be performed either manually or using an automated software.

XV. Angiogenesis: Plate Coating

Plate Coating Components (Sold Separately)

- Matrigel® Basement Membrane Matrix, Phenol Red-Free (Corning Catalog No. 356237, or similar)

1. Thaw the bottle of Matrigel® Basement Membrane Matrix overnight at 4℃ on ice.
2. Pre-cool the desired culture plate and maintain the culture plate on ice during the coating process.

NOTE: For best results, use of a 48-well or 96-well culture plate is highly recommended.

3. Using pre-cooled pipette tips, transfer 200 µl/cm^2 of the Matrigel® to the pre-cooled culture vessel (for example, transfer 150 µl of the Matrigel® to each well of a 48-well plate or transfer 75 µl of the Matrigel® to each well of a 96-well plate).
4. Aliquot the remaining Matrigel® in 1 mL aliquots into sterile, pre-cooled 1.5 mL tubes and store immediately in a -20℃ freezer. Avoid multiple freeze thaws. Do not store in a frost-free freezer.
5. Incubate the plate at room temperature for at least ten minutes.
6. After room temperature incubation, incubate the plate in a humidified 37℃ incubator with 5% CO_2 for 30.

NOTE: When the coating procedures have been completed, the cells must be plated immediately. Do not store the coated flasks, petri dishes or cover slips for later use.

XVI. Angiogenesis: Protocol

Angiogenesis Components (Sold Separately)

- One pre-screened HUVEC cell product – (proliferating) (Lonza Catalog No. C2517AS, C2519AS, or similar)
- One Endothelial Growth Medium-2 (EGM™-2 Medium) – 500 ml (prepared as described in Section IX [Preparation of Culture Media, Page 7)
- Culture vessel pre-coated with Matrigel® Basement Membrane Matrix (prepared as

described in Section XV [Angiogenesis: Plate Coating, Page 11)

NOTE: For differentiation of these cells, cells must be cultured at 37℃±1℃, 5% CO_2, 90%±2% humidity.

1. The recommended seeding density when plating HUVEC for angiogenesis is 65,000-80,000 cells/cm^2.
2. To set up culture vessels, calculate the number of Matrigel® Basement Membrane Matrix pre-coated vessels needed based on the recommended seeding density and the surface area of the vessels being used.
3. Add the appropriate amount of growth medium (EGM™-2 Medium) containing any experimental angiogenesis inhibitors/promoters at 2X concentration to the vessels (0.5 ml/3 cm^2) and allow the vessels to equilibrate in a 37℃±1℃, 5% CO_2, 90%±2% humidity incubator for at least 30 minutes. (For example, add 125 µl of culture media containing any angiogenesis inhibitors/promoters at 2X concentration per well of a 48-well plate or plate 75 µl of culture media containing any angiogenesis inhibitors/promoters at 2X concentration per well of a 96-well plate).

NOTE: Experimental angiogenesis inhibitors/promoters must be at 2X concentration as the solution will be further diluted with cell suspension upon plating. For control samples, add the necessary amount of media without the addition of inhibitors/promoters. For positive inhibition controls Suramin (Sigma Catalog No. S2671, or similar) can be added at 30 µM and 60 µM (15 µM and 30 µM after final dilution)

4. Subculture cells according to Steps 1 through 17 of Section XII (Subculturing, Page 8).

NOTE: For best results, cells should be used in early passages.

5. Further dilute the cell suspension with fresh growth medium (EGM™-2 Medium) to a final concentration of 400,000 cells/ml.
6. Plate the cell suspension at 0.5 ml/3 cm^2 into the previously prepared Matrigel® Basement Membrane Matrix pre-coated vessels containing angiogenesis inhibitor/promoter containing medium at 2X concentration. (For example, plate 125 µl of cell suspension per well of a 48-well plate or plate 75 µl of cell suspension per well of a 96-well plate).
7. Tube formation typically starts within 4 hours after plating cells. Complete tube formation typically takes 12 to 16 hours, and tubule disruption will typically occur 16 to 18 hours after plating. Analysis of tube formation should occur within the first 16 hours after cell plating. Tubule formation can be visualized directly by microscopy or by first staining with a live cell staining dye such as Calcein AM.

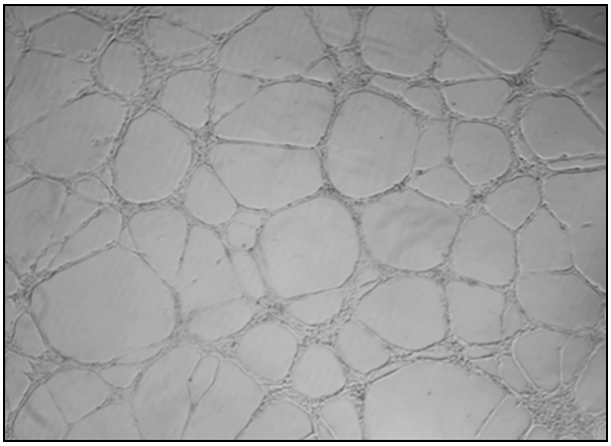

Figure 1. Tube formation of HUVEC plated at 50,000 cells per well of a Matrigel® coated, 48-well plate in Lonza EGM™-2 Medium after 16 hours in culture at 5X magnification.

General Notes on Angiogenesis:

- In theory, all endothelial cells should have the capability to undergo angiogenesis in culture under the proper conditions. This protocol may be applicable to other, non-HUVEC, endothelial cells so long as the correct media is utilized, however, the conditions may need to be optimized depending on the cell type.
- The degree of angiogenesis and response to angiogenesis inhibitors/promoters may be widely variable depending on the cell type, the donor, and the passage number.
- Cell density is critical for angiogenesis. Too few cells will yield incomplete tubes while too many cells will yield large areas of cell clusters or monolayers.

XVII. Ordering Information

Cryopreserved Normal Endothelial Cells

Cat. No.	Product	Description
CC-2535	HAEC	≥500,000 cells
CC-2585	HCAEC	≥500,000 cells
CC-2545	HIAEC	≥500,000 cells
CC-2530	HPAEC	≥500,000 cells
CC-2519	HUVEC, Pooled Donors, Cryopreserved in EGM™	≥500,000 cells
C2519A	HUVEC, Pooled Donors, Cryopreserved in EGM™-2	≥500,000 cells
C2519AS	HUVEC, Prescreened, Pooled Donors, Cryopreserved in EGM™-2	≥500,000 cells
00191027	HUVEC-XL, Pooled Donors, Cryopreserved in EGM™-2	≥10,000,000 cells
CC-2517	HUVEC, Single Donor, Cryopreserved in EGM™	≥500,000 cells
C2517A	HUVEC, Single Donor, Cryopreserved in EGM™-2	≥500,000 cells
C2517AS	HUVEC, Prescreened, Single Donor, Cryopreserved in EGM™-2	≥500,000 cells
CC-2935	HUVEC, Single Donor, Cryopreserved in EGM™-PLUS	≥500,000 cells
CC-7016	HMVEC-Bd	≥500,000 cells
CC-7030	HMVEC-C	≥500,000 cells
CC-2811	HMVEC-DBlAd	≥500,000 cells
CC-2543	HMVEC-DAd	≥500,000 cells
CC-2516	HMVEC-DNeo	≥500,000 cells
CC-2505	HMVEC-DNeo	≥500,000 cells
CC-2813	HMVEC-DBlNeo	≥500,000 cells
CC-2810	HMVEC-DLyAd	≥500,000 cells
CC-2812	HMVEC-DLyNeo	≥500,000 cells
CC-2527	HMVEC-L	≥500,000 cells
CC-2814	HMVEC-LLy	≥500,000 cells
CC-2564	UtMVEC-Myo	≥500,000 cells

Proliferating cultures are also available in a variety of culture vessels including flasks and well plates. For more information regarding proliferating cultures, including for catalog numbers, please contact Lonza Scientific Support.

Cryopreserved Diseased Endothelial Cells

Cat. No.	Product	Description
CC-2919	D-HAEC Diabetic Type I	≥500,000 cells
CC-2920	D-HAEC Diabetic Type II	≥500,000 cells
CC-2921	D-HCAEC Diabetic Type I	≥500,000 cells
CC-2922	D-HCAEC Diabetic Type II	≥500,000 cells
CC-2923	D-HPAEC Diabetic Type I	≥500,000 cells
CC-2924	D-HPAEC Diabetic Type II	≥500,000 cells
CC-2927	D-HMVEC-C Diabetic Type I	≥500,000 cells
CC-2928	D-HMVEC-C Diabetic Type II	≥500,000 cells
CC-2929	D-HMVEC-Dad Diabetic Type I	≥500,000 cells
CC-2930	D-HMVEC-Dad Diabetic Type II	≥500,000 cells

Proliferating cultures are also available in a variety of culture vessels including flasks and well plates. For more information regarding proliferating cultures, including for catalog numbers, please contact Lonza Scientific Support.

Endothelial Growth Media: Without Exogenous VEGF (Sold Separately)

Cat. No.	Product	Description
CC-3124	EGM™ BulletKit™ Medium	500 ml EBM™ Basal Medium plus CC-4133 SingleQuots™ Kit to formulate EGM™ Medium (growth medium)
CC-3121	EBM™ Basal Medium	Endothelial basal medium (500 ml)
CC-3129	EBM™ Basal Medium - PR Free	Endothelial basal medium (500 ml); Phenol Red Free
CC-4133	EGM™ SingleQuots™ Kit	Formulates 500 ml of EBM™ Basal Medium to EGM™ Growth Medium; contains BBE, 2.0 ml; Ascorbic Acid, 0.5 ml; Hydrocortisone, 0.5 ml; hEGF, 0.5 ml; FBS, 10.0 ml; GA, 0.5 ml
CC-5035	EGM™-PLUS BulletKit™ Medium	500 ml EBM™-PLUS Basal Medium plus CC-4542 SingleQuots™ Kit to formulate EGM™-PLUS Medium (growth medium)
CC-5036	EBM™-PLUS Basal Medium	Endothelial basal medium-PLUS (500 ml); Phenol Red Free
CC-4542	EGM™-PLUS SingleQuots™ Kit	Formulates 500 ml of EBM™-PLUS Basal Medium to EGM™-PLUS Growth Medium; contains EnGS, 1.0 ml; L-Glutamine, 25.0 ml; Ascorbic Acid, 0.5 ml; Hydrocortisone Hemisuccinate, 0.5 ml; hEGF, 0.5 ml; Heparin 0.5 ml; FBS, 10.0 ml; GA, 0.5 ml

EBM™ Phenol Red Free (CC-3129) can be used as a phenol red-free alternative to either EBM™ Basal Medium (CC-3121) or EBM™-2 Basal Medium (CC-3156), however, the appropriate SingleQuots™ Kit must be added for endothelial cell growth (sold separately).

Endothelial Growth Media: With VEGF
(Sold Separately)

Cat. No.	Product	Description
CC-3162	EGM™-2 BulletKit™ Medium	500 ml EBM™-2 Basal Medium plus CC-4176 SingleQuots™ Kit to formulate EGM™-2 Medium (growth medium)
CC-3156	EBM™-2 Basal Medium	Endothelial basal medium-2 (500 ml)
00190860	EBM™-2 Basal Medium	Endothelial basal medium-2 (1 L)
CC-4176	EGM™-2 SingleQuots™ Kit	Formulates 500 ml of EBM™-2 Basal Medium to EGM™-2 Growth Medium; contains hEGF, 0.5 ml; VEGF, 0.5 ml; R3-IGF-1, 0.5 ml; Ascorbic Acid, 0.5 ml; Hydrocortisone, 0.2 ml; hFGF-β, 2.0 ml; Heparin 0.5 ml; FBS, 10.0 ml; GA, 0.5 ml
CC-3202	EGM™-2MV BulletKit™ Medium	500 ml EBM™-2 Basal Medium plus CC-4147 SingleQuots™ Kit to formulate EGM™-2MV Medium (growth medium)
CC-4147	EGM™-2MV SingleQuots™ Kit	Formulates 500 ml of EBM™-2 Basal Medium to EGM™-2MV Growth Medium; contains hEGF, 0.5 ml; VEGF, 0.5 ml; R3-IGF-1, 0.5 ml; Ascorbic Acid, 0.5 ml; Hydrocortisone, 0.2 ml; hFGF-β, 2.0 ml; FBS, 25.0 ml; GA, 0.5 ml

EBM™ Phenol Red Free (CC-3129) can be used as a phenol red-free alternative to either EBM™ Basal Medium (CC-3121) or EBM™-2 Basal Medium (CC-3156), however, the appropriate SingleQuots™ Kit must be added for endothelial cell growth (Sold Separately).

Subculturing Reagents: (Sold Separately)

Cat. No.	Product	Description
CC-5034	ReagentPack™	Provides necessary components for subculture of endothelial cells; contains Trypsin/EDTA Solution, 100 ml; Trypsin Neutralizing Solution (TNS), 100 ml; HEPES Buffered Saline Solution, 100 ml
CC-5012	Trypsin/EDTA Solution	100 ml
CC-5002	Trypsin Neutralizing Solution (TNS)	100 ml
CC-5022	HEPES-BSS (1X)	HEPES Buffered Saline Solution (1X) (100 ml)
CC-5024	HEPES-BSS (1X)	HEPES Buffered Saline Solution (1X) (500 ml)

XVIII. Product Warranty

Cultures have a finite lifespan *in vitro.*

Lonza guarantees the performance of Clonetics™ cells only if appropriate Clonetics™ media and reagents are used exclusively and the recommended storage and use protocols are followed. Any modifications made to the recommended cell systems including the use of alternative media, reagents or protocols, will void cell and media performance guarantees. If you need assistance in selecting the appropriate media, reagents, or protocol, please contact Lonza Scientific Support.

1. Clonetics™ HAEC, HCAEC, HPAEC, HUVEC, HMVEC-D, HMVEC-L, and UtMVEC-Myo cryopreserved cultures are assured for experimental use for fifteen population doublings. Cryopreserved HMVEC-DBl, HMVEC-DLy and HMVEC-LLy are assured for experimental use for at least twelve population doublings. HIAEC, HMVEC-Bd, and HMVEC-C cryopreserved cultures are assured for experimental use for ten population doublings. D-HAEC, D-HCAEC, D-HPAEC, D-HMVEC-C, and D-HMVEC-D cryopreserved cultures are assured to reach ≥90% confluence in the first passage from cryopreservation. Total proliferation doublings obtained in the first two passages from cryopreservation are provided For Information Only (FIO).
2. Clonetics™ Normal Endothelial Proliferating Cultures are assured for experimental use for at least five population doublings. Clonetics™ Diseased Endothelial Proliferating Cultures are assured for experimental use for one passage upon receipt.
3. Additional population doublings and subcultures may be possible, but growth rate, biological responsiveness and function deteriorate with subsequent passage.
4. Endothelial cells can become irreversibly contact-inhibited if allowed to reach confluence. To avoid the loss of your cells and forfeiture of your warranty, subculture cells before they reach 80% confluence.

When placing an order or for Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or want to speak with Scientific Support, you may contact Lonza by web, e-mail, telephone, fax or mail
(See page 1 for details).

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* diagnostic procedures.

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th ed. If you require further information, please contact your site safety officer or Scientific Support.

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