

GFP-Human iPSC-Derived Endothelial Precursor Cells (iPSCEPCs)

Catalog Number: IPSCEP002

Cell Quantity: 500,000 cells per vial ($\geq 90\%$ viability post-thaw)

Product Format: Cryopreserved in Serum-Free Cryopreservation Medium

PRODUCT DESCRIPTION

Human iPSC-EPCs (Catalog # IPSCEP001) are highly pure, cryopreserved endothelial precursor cells derived from induced pluripotent stem cells (iPSCs) using a directed, serum-free, GSK-3 β and TGF- β inhibitor-based differentiation protocol (CHIR99021 + SB431542). These cells represent an intermediate stage between mesodermal progenitors and mature endothelial cells, exhibiting robust proliferative capacity and the ability to differentiate into mature endothelial cells (arterial, venous, and lymphatic subtypes).

Source: Normal human iPSC line (fully characterized, pathogen-free, normal karyotype)

EXPRESSION MARKER PROFILE

All markers validated by immunocytochemistry (ICC), flow cytometry ($\geq 85\%$ purity), and RT-qPCR.

Marker Category	Marker Name	Expression	Cellular Localization
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Endothelial Lineage Markers	CD31 (PECAM-1)	Positive ($\geq 85\%$)	Cell membrane / intercellular junctions
	VE-Cadherin (CD144)	Positive ($\geq 80\%$)	Adherens junctions
	von Willebrand Factor (vWF)	Positive (low–moderate)	Weibel-Palade bodies (cytoplasmic)
	Endoglin (CD105)	Positive	Cell membrane
	CD34	Positive ($\geq 70\%$)	Cell surface glycoprotein
Endothelial Precursor Markers	KDR (VEGFR2, CD309)	High positive ($> 90\%$)	Receptor tyrosine kinase (membrane)
	TIE2 (CD202b)	Positive	Angiopoietin receptor
	ETV2 (ER71)	Positive (Nuclear)	Master transcription factor for endothelial specification
	FLI1	Positive (Nuclear)	ETS family transcription factor
Mesodermal Progenitor Markers	BRACHYURY (T)	Low ($< 5\%$)	Early mesoderm (downregulated)
Marker Category	Marker Name	Expression	Cellular Localization

	MESP1	Negative	Cardiac mesoderm (excluded)
Pluripotency & Off-target Exclusion	OCT4	Negative ($\leq 0.5\%$)	Confirms absence of residual iPSCs
	NANOG	Negative	Absence of pluripotency
	SOX17	Negative	Excludes endodermal contamination
	PAX6	Negative	Excludes neural contamination
	α -SMA	Negative ($< 2\%$)	Excludes smooth muscle / pericyte contamination
Proliferation Markers	Ki67	$> 50\%$ of cells	Nuclear (proliferative fraction)
Mature Endothelial Markers (low/absent)	ICAM-2	Low	Upregulated upon maturation
	Podoplanin (PDPN)	Negative	Lymphatic-specific (absent)
	NOS3 (eNOS)	Low/absent	Induced during shear stress maturation

STORAGE & HANDLING

Parameter	Condition
Immediate storage (upon arrival)	Liquid nitrogen vapor phase (–150°C to –190°C)
Short-term storage (not recommended)	–80°C for ≤1 week (will significantly reduce viability)
Thawing medium	EPC Thawing Medium (Cat # MEDEPC-T1)
Plating density	15,000–25,000 cells/cm ²
Coating requirement	Smooth Coat Solution
Recommended expansion medium	EPC Expansion Medium (Cat # MEDEPC -X1)

THAWING & CULTURE PROTOCOL

1. Thaw vial in 37°C water bath (<2 minutes) until a small ice crystal remains.
2. Transfer cells dropwise into 10 mL pre-warmed **EPC Thawing Medium**.
3. Centrifuge at 300×g for 5 minutes.
4. Resuspend in **EPC Expansion Medium** and plate onto Smooth Coat Solutioncoated surface.
5. Change medium every 24–48 hours; cells reach confluence in 4–6 days.
6. Passage at 80–90% confluence using **Cell Detachment Solution**(3–5 minutes at 37°C).
7. Split ratio: 1:3 to 1:4 for maintenance.

8. **Critical note:** Do not overgrow; confluent monolayers rapidly lose EPC phenotype.

FUNCTIONAL & DIFFERENTIATION CAPACITY

Differentiation Condition	Endpoint	Expected Marker Expression
Mature endothelial (VEGF + FGF-2 + 5% FBS for 7 days)	Day 7–10	High CD31 (>95%), VE-Cadherin (tight junctions), eNOS+
Arterial specification (VEGF + NOTCH agonist DLL4)	Day 7	EFNB2+, NR2F2+ (COUP-TFII low)
Venous specification (NRP1 inhibition)	Day 7	NR2F2+, EFNB2 low
Tube formation	6–18 hours	Capillary-like networks (quantified by total tube length)
Acetylated LDL uptake	4 hours	Dil-Ac-LDL+ (functional scavenger receptor activity)

QUALITY CONTROL

Test	Specification	Method
Viability post-thaw	≥90%	Trypan blue exclusion
Purity (% CD31+ / VE-Cadherin+)	≥85%	Flow cytometry
Purity (% KDR+/CD34+)	≥80%	Flow cytometry
Mycoplasma	Negative	qPCR / culture
Bacterial / Fungal sterility	Negative	14-day culture
Pluripotency marker (OCT4, NANOG)	<0.5%	Flow cytometry / ICC
Endotoxin	<0.5 EU/mL	LAL assay
Karyotype	Normal (46,XX or 46,XY)	G-banding (every 5 lots)
Tube formation assay	Pass (forms capillary-like structures)	Assay
Ac-LDL uptake	Pass (>80% uptake)	Fluorescence microscopy

APPLICATIONS

- **Vascular disease modeling:** Hereditary hemorrhagic telangiectasia (HHT), cerebral cavernous malformation (CCM), diabetic vasculopathy
- **Angiogenesis & tube formation assays** (high-throughput screening)
- **Co-culture with iPSC-neurons or cardiomyocytes** (neurovascular unit / cardiac vascularization)
- **Tissue engineering** (vascularized organoids, microfluidic chips)
- **Drug screening** (pro-angiogenic or anti-angiogenic compounds)
- **BBB (Blood-Brain Barrier) models** (co-cultured with pericytes and astrocytes)

LIMITATIONS & PRECAUTIONS

- **For research use only.** Not for human therapeutic, diagnostic, or clinical use.
- EPCs exhibit limited expansion potential: **passage 8–10 maximum** before senescence.
- Avoid trypsin (use Cell Detachment Solution to prevent receptor cleavage).
- Do not freeze-thaw more than once after receiving the vial.
- Use low-passage cells ($\leq P5$) for tube formation and in vivo studies.
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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Poor attachment	Coating inadequate	Fresh collagen I + fibronectin; allow coating to dry partially

Slow proliferation	Low VEGF/FGF-2 activity	Check growth factor batch; replace medium every 24h
Problem	Possible Cause	Solution
Loss of CD31 expression	Overconfluence / high passaging	Passage at <90%; use cells $\leq$ P6
Spontaneous differentiation	FBS lot variability	Use endothelial-qualified FBS ($\leq$ 5%)
Cell detachment during media change	Shear stress	Pipette gently along vessel wall

ORDERING INFORMATION & RELATED PRODUCTS

Product	Catalog Number	Size
Human iPSC-EPCs (500K cells)	IPSCEP001	1 vial
EPC Thawing Medium	MEDEPC-T1	50 mL
EPC Expansion Medium	MEDEPC-X1	500 mL
Coating Kit	ECM-EPC1	1 kit (5 coatings, T75)
Mature Human iPSC-Endothelial Cells	IPSCEC002	500K cells



Endothelial Tube Formation Assay Kit

ANGIO-1

96-well

Dil-Ac-LDL

LDL-DII-1

200 µg