Vascular cell culture under physiological oxygen tensions – implications for cellular redox homeostasis

PromoCell®

Application note

Endothelial cell behavior under physiological hypoxia conditions

Endothelial cells (ECs) line the inner surfaces of all blood and lymphatic vessels, forming a thin layer called the endothelium. Processes such as angiogenesis and vasculogenesis, hemostasis, vasomotor tone and immune and inflammatory responses depend on the capacities of ECs [9]. In addition, ECs play a role in numerous pathologic states, e.g. human vascular diseases and chronic diseases associated with oxidative stress [5].

Oxidative stress emerges from exceeding the cells' antioxidant capacity to handle reactive oxygen species (ROS). ROS are formed by incomplete oxidation or reduction of O_2 during processes such as aerobic metabolism or electron transport and can damage proteins, lipids and DNA [4, 11]. Oxygen homeostasis is therefore a very important process in all nucleated cells, illustrating the delicate correlation between O_2 availability and its manifestation in cellular responses, e.g. angiogenesis, proliferation and differentiation [6, 7]. Hypoxic microenvironments have been shown to promote angiogenesis in tumor tissues, immunosuppression and metastatic progression in cancer by hypoxia-inducible factor (HIF) activation [1, 7]. HIF induction is one of the mechanisms that is known to directly correlate with hypoxic states and prompt the transcription of angiogenic growth factors such as VEGF (vascular endothelial growth factor) [6].

Transcription factor Nrf2 (NF-E2-related factor 2) is another factor that that is known to regulate the expression of a very large number of genes involved in processes such as cytoprotection or lipid and carbohydrate metabolism, in both unstressed homeostatic and under stressed and perturbed conditions [10].

Whereas the physiological oxygen levels present in the vascular microenvironment *in vivo* range from 3 – 13% [12], common cell culture systems using traditional incubators maintain an atmospheric oxygen level of 18 – 21%. Endothelial cells cultured *in vitro* with an oxygen level of 5% revealed altered regulation of Nrf2 downstream targets compared to ECs cultured at atmospheric oxygen levels (see Fig. 3) [2]. It is important to mention that most research results have been obtained in conventional cell culture systems with an atmospheric oxygen level used as "normoxia" control, which does not correspond to the "normal" oxygen levels *in vivo* [2]. Rather, the atmospheric oxygen level corresponds to a hyperoxic environment (see Fig. 1). Chapple *et al.* examined the differences between these culturing conditions and analyzed their impact on endothelial cell behavior (see Figs. 2 and 3).

In general, it is advisable to consider how oxygen levels influence cell behavior and keep these implications in mind when working with redox-sensitive processes, to choose the appropriate culturing conditions.
However, the goal is to use a model that is as realistic and reliable as possible in order to mimic fundamental *in vivo* conditions present in process regulation and mechanisms and prevent from measuring artefacts (see Fig. 2 and Tab. 1).

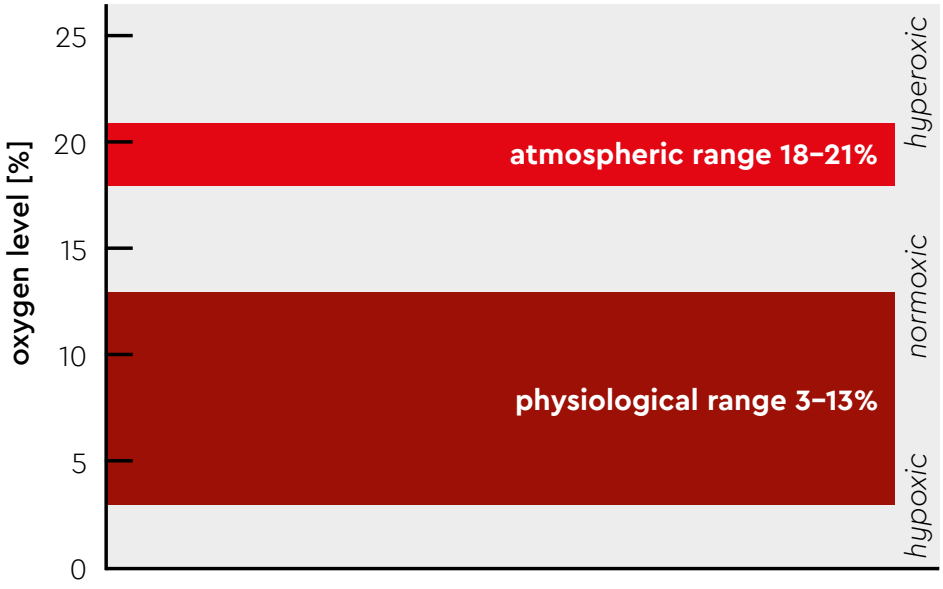
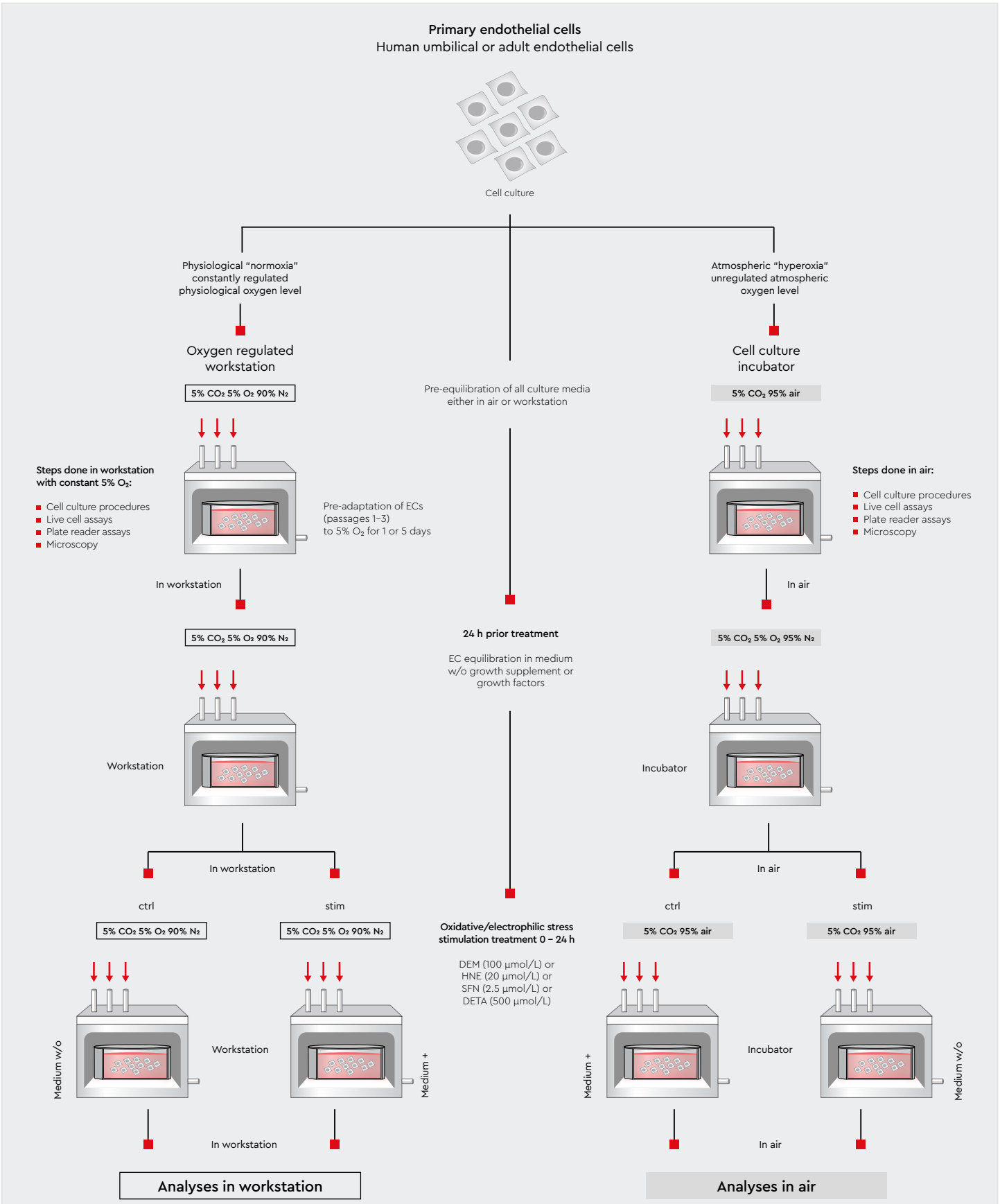


Fig. 1: Oxygen level conditions: *Endothelial cells reside in a physiological O_2 environment in vivo, whereas under common cell culture conditions cells are normally exposed to atmospheric O_2 levels. For more detailed information, see Chapple et al. [2].*

Endothelial cell culture under physiological (5% O_2) vs. atmospheric oxygen conditions (18–21% O_2)



DEM: diethylmaleate, DETA: NO donor, ECs: endothelial cells, ECGS: endothelial cell growth supplement, ECM: endothelial cell medium, FCS: fetal calf serum, HNE: 4-hydroxynonenal, NCS: newborn calf serum, SFN: sulforaphane, ctrl: control, stim: stimulated, w/o: without

Fig. 2: Endothelial cell culture under physiological vs. atmospheric oxygen conditions: *Description and comparison of the respective culturing methods with emphasis on changes in the oxygen level. For more detailed information, see Chapple et al. [2].*

	Experimental control conditions	
	Physiological O_2 level (5%)	Atmospheric O_2 level (18 – 21%)
Advantages	■ No additional oxygen tensions during cell culture procedures ■ Closer to *in vivo* conditions ■ Generate more reliable data ■ Better insights into physiological processes ■ Better clinical translation	■ Standard cell culture equipment
Disadvantages	■ Oxygen regulated cell culture system required	■ Artefactual observations possible ■ Additional oxygen tensions during cell culture procedures for ECs cultured under hypoxic conditions (passaging, live cell assays in air)

Table 1: Comparison of the advantages and the disadvantages of the two different methodological control conditions: *Working with an O_2-regulated workstation in contrast to unregulated atmospheric O_2 cell culture conditions.*

Changes in Nrf2 pathway regulation in endothelial cell culture under physiological oxygen conditions (5% O_2)

It has emerged, that the redox sensitive Nrf2/Keap1 (NF-E2-related factor 2/Kelch-like ECH-associated protein 1) pathway acts as a potent regulator of health and disease [10]. Experiments with human primary ECs (HUVECs, HCAECs, PromoCell) adapted to physiological O_2 conditions (5%) showed a significantly altered Nrf2 regulation and corresponding downstream targets compared to atmospheric oxygen cell culture conditions [2]. Whereas cell ultrastructure, viability, basal redox status and HIF1-α were only minimally affected, the induced expression of Nrf2 downstream targets as HO-1 and NQO1 was attenuated due to Bach1 upregulation [2] (see Fig. 3).

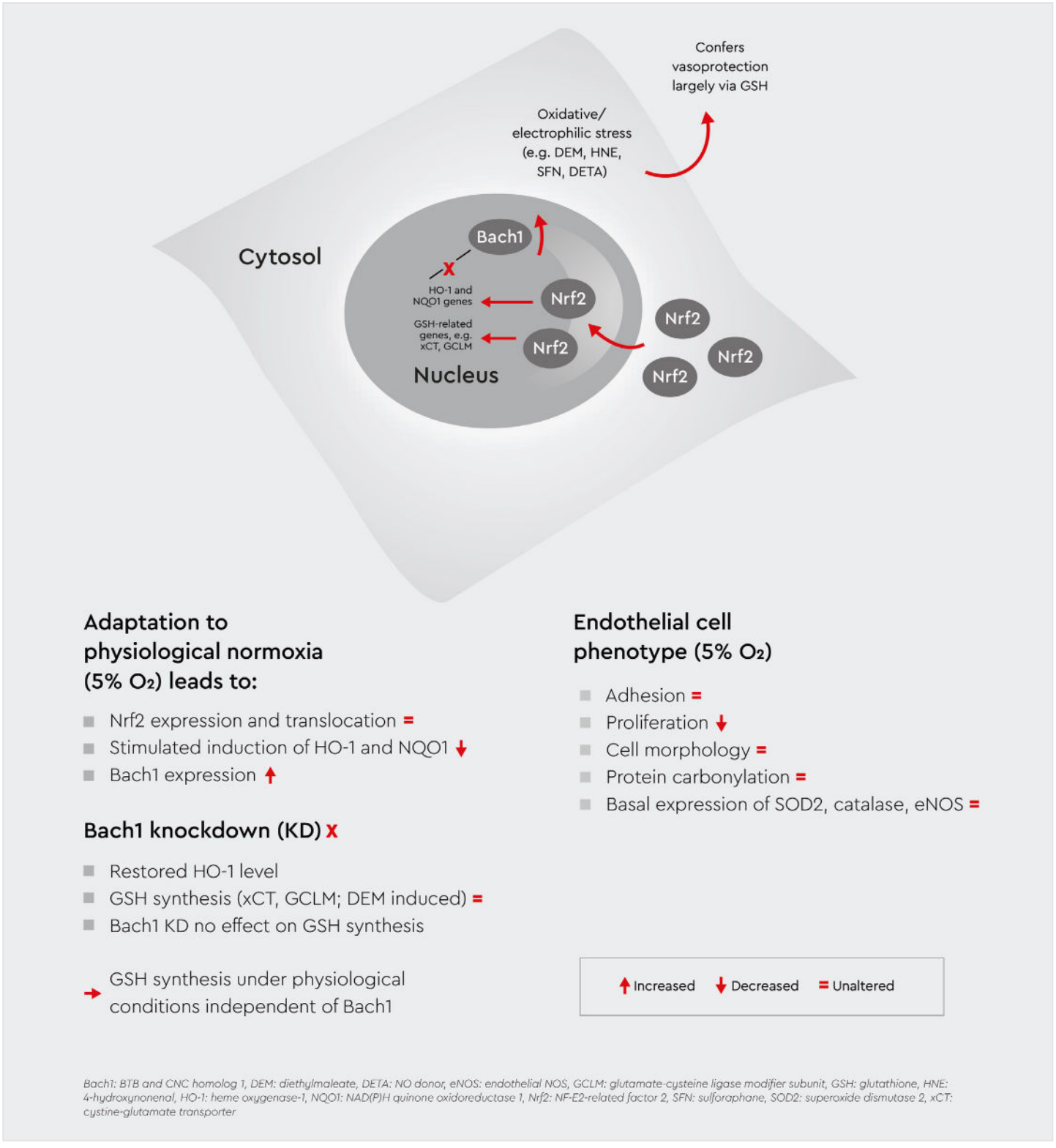


Fig. 3: Differential regulation of Nrf2-targeted genes in human endothelial cells adapted to physiological O_2 levels encountered in vivo compared to atmospheric O_2 levels. *Illustrated and simplified according to Chapple et al. [2].*

Conclusion

Culturing endothelial cells at physiological O_2 levels provides a more accurate model by reducing artefactual observations *in vitro* as demonstrated here by the altered regulation of selected Nrf2 target genes by Bach1 [2]. This makes it possible to generate more reliable data from *in vitro* assays and translate them better into medical applications such as treatment of vascular diseases, wound healing, cancer or stroke [6, 8].

Products

Endothelial cell type	Size	Catalog number
Human Umbilical Vein Endothelial Cells (HUVEC) pooled	500.000 cryopreserved cells 500.000 proliferating cells	C-12203, C-12253
Human Coronary Artery Endothelial Cells (HCAEC)	500.000 cryopreserved cells 500.000 proliferating cells	C-12221, C-12222
Endothelial Cell Growth Medium (Ready-to-use)	500 ml	C-22010
Endothelial Cell Growth Medium MV (Ready-to-use)	500 ml	C-22020

Related products

Endothelial cell type	Size	Catalog number
Human Umbilical Vein Endothelial Cells (HUVEC) single donor	500.000 cryopreserved cells 500.000 proliferating cells	C-12200, C-12250
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, single donor	500.000 cryopreserved cells 500.000 proliferating cells	C-12206, C-12207
Human Umbilical Vein Endothelial Cells (HUVEC) isolated in Growth Medium 2, pooled	500.000 cryopreserved cells 500.000 proliferating cells	C-12208, C-12209
Human Umbilical Vein Endothelial Cells (HUVEC) pre-screened	500.000 cryopreserved cells 500.000 proliferating cells	C-12205, C-12255
Human Umbilical Artery Endothelial Cells (HUAEC)	500.000 cryopreserved cells 500.000 proliferating cells	C-12202, C-12252
Human Aortic Endothelial Cells (HAoEC)	500.000 cryopreserved cells 500.000 proliferating cells	C-12271, C-12272
Human Pulmonary Artery Endothelial Cells (HPAEC)	500.000 cryopreserved cells 500.000 proliferating cells	C-12241, C-12242
Human Saphenous Vein Endothelial Cells (HSaVEC)	500.000 cryopreserved cells 500.000 proliferating cells	C-12231, C-12232
Human Dermal Microvascular Endothelial Cells (HDMEC) juvenile foreskin	500.000 cryopreserved cells 500.000 proliferating cells	C-12210, C-12260

Endothelial cell type	Size	Catalog number
Human Dermal Microvascular Endothelial Cells (HDMEC) adult donor	500.000 cryopreserved cells 500.000 proliferating cells	C-12212, C-12262
Human Dermal Microvascular Endothelial Cells (HDMEC) pre-screened	500.000 cryopreserved cells 500.000 proliferating cells	C-12215, C-12265
Human Dermal Blood Endothelial Cells (HDBEC) juvenile foreskin	500.000 cryopreserved cells 500.000 proliferating cells	C-12211, C-12214
Human Dermal Blood Endothelial Cells (HDBEC) adult donor	500.000 cryopreserved cells 500.000 proliferating cells	C-12225, C-12226
Human Dermal Lymphatic Endothelial Cells (HDLEC) juvenile foreskin	500.000 cryopreserved cells 500.000 proliferating cells	C-12216, C-12218
Human Dermal Lymphatic Endothelial Cells (HDLEC) adult donor	500.000 cryopreserved cells 500.000 proliferating cells	C-12217, C-12219
Human Cardiac Microvascular Endothelial Cells (HCMEC)	500.000 cryopreserved cells 500.000 proliferating cells	C-12285, C-12286
Human Pulmonary Microvascular Endothelial Cells (HPMEC)	500.000 cryopreserved cells 500.000 proliferating cells	C-12281, C-12282
Human Uterine Microvascular Endothelial Cells (HUtMEC)	500.000 cryopreserved cells 500.000 proliferating cells	C-12295, C-12296
Endothelial Cell Growth Medium 2 (Ready-to-use)	500 ml	C-22011
Endothelial Cell Growth Medium MV 2 (Ready-to-use)	500 ml	C-22022
Endothelial Cell Growth Medium Kit	500 ml	C-22110
Endothelial Cell Growth Medium 2 Kit	500 ml	C-22111
Endothelial Cell Growth Medium MV Kit	500 ml	C-22120
Endothelial Cell Growth Medium MV 2 Kit	500 ml	C-22121
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912

References

1. Bishop T and Ratcliffe PJ (2015) HIF Hydroxylase Pathways in Cardiovascular Physiology and Medicine. Circ Res 117:65–79.
2. Chapple SJ, Keeley TP, Mastronicola D, Arno M, Vizcay-Barrena G, Fleck R, Siow RC, Mann GE (2016) Bach1 differentially regulates distinct Nrf2-dependent genes in human venous and coronary artery endothelial cells adapted to physiological oxygen levels. Free Radic Biol Med 92:152–162.
3. Giaccia AJ, Simon MC, Johnson R (2004) The biology of hypoxia: the role of oxygen sensing in development, normal function, and disease. Genes Dev 18:2183–2194.
4. Ishii T, Itoh K, Ruiz KE, Leake DS, Unoki H, Yamamoto M and Mann GE (2004) Role of Nrf2 in the Regulation of CD36 and Stress Protein Expression in Murine Macrophages: Activation by Oxidatively Modified LDL and 4-Hydroxynonenal. Circ Res 94:609- 616.
5. Kanninen KM, Pomeshchik Y, Leinonen H, Malm T, Koistinaho J, Levonen AL (2015) Applications of the Keap1-Nrf2 system for gene and cell therapy. Free Radic Biol Med 88:350–361
6. Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ_, Garcia JG, Semenza GL (2005) Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. Blood 105:659–669.
7. Rankin EB, Giaccia AJ (2016) Hypoxic control of metastasis. Science 352:175–180.
8. Semenza GL (2012) Hypoxia-inducible factors in physiology and medicine. Cell 148:399–408.
9. Sumpio BE, Riley JT, Dardik A (2002) Cells in focus: endothelial cell. Int J Biochem Cell Biol 34:1508–1512.
10. Suzuki T, Yamamoto M (2015) Molecular basis of the Keap1-Nrf2 system. Free Radic Biol Med 88:93–100.
11. Tebay LE, Robertson H, Durant ST, Vitale SR, Penning TM, Dinkova-Kostova AT, Hayes JD (2015) Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. Free Radic Biol Med 88:108–146.
12. Ward JP (2008) Oxygen sensors in context. Biochim Biophys Acta 1777:1–14.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 96 03 33 (toll free)
Fax: 0800 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

11/2023