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DA3563 Bovine ECGFpro1 (complete for blood ECs)

Quantity: 6 mg

Background: Endothelial cell growth factor (ECGF) is an extract of bovine brain containing growth promoting factors for vascular endothelial cells of mammalian origin. Endothelial cell growth factor is prepared using a modification of the method of Maciag, et al. (1979) lyophilized from a sterile solution containing NaCl and streptomycin sulfate.

ECGFpro1 is supplemented with recombinant human VEGF165 (corresponding to 10 ng/ml) a concentration sufficient for the cultivation of blood vascular endothelial cells like HUVEC and HMVEC.

Endothelial cells from human umbilical vein (HUVEC) can be established as primary cultures by traditional methods. The serial propagation of these cells has proved to be difficult. The long-term propagation of these cells in vitro can be achieved with an extract prepared from bovine brain. The introduction of a fibronectin or collagen matrix to the cell culture system allows cultivating endothelial cells at clonal densities. With ECGF, the FCS requirement can be reduced. Heparin potentiates the mitogenic activity of crude preparations of ECGF. ECGF has also been reported to eliminate the need for feeder cells in the clonal growth of hybridomas and other cell types.

Source: Brain, Bovine brain (BSE-free tested!)

Format:
State: Sterile, lyophilized, Freeze dried powder
Purity: Crude extract.
Buffer System: H2O without preservative.
Reconstitution: To obtain a stock solution reconstitute the contents of the vial in 2 ml of prewarmed (37 °C) sterile PBS or water. Gently rotate the vial until the contents are dissolved. This stock solution may be further diluted in sterile tissue culture media to obtain the desired working concentrations. Although the stock solution can be added aseptically to sterile tissue culture medium, it is recommended that medium containing diluted product is aseptically filtered prior to use.
The ECGF + VEGF-A are sufficient for 500 ml growth medium.

Applications: **Biological activity/ Working concentration:** Optimum concentration for human umbilical vein endothelial cells (HUVEC) range from 50-200 µg/ml, optimal concentration with heparin (50 µg/ml) is about 12 µg/ml.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Description: Endothelial cell growth factor (ECGF) is an extract of bovine brain containing growth promoting factors for vascular endothelial cells of mammalian origin. ECGF has also been reported to be beneficial as a media supplement for the fusion and growth of hybridoma cells in monoclonal antibody production. Endothelial cell growth factor is prepared using a modification of the method of Maciag, et al. (1979) lyophilized from a sterile solution containing NaCl and streptomycin sulfate.

ECGFpro1 is supplemented with recombinant human VEGF165 (corresponding to 10 ng/ml) a concentration sufficient for the cultivation of blood vascular endothelial cells like HUVEC and HMVEC.
Species specificity: Bovine ECGF is effective on Mouse, Bovine and Human cells.

Add. Information:

Additional Factor: rh-VEGF165 (final concentration: 10ng/ml)

Storage:

Store lyophilized at 2-8°C for 6 months or at -20°C long term.
After reconstitution store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C long term.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:

1. Maciag T, Hoover GA, Weinstein R. High and low molecular weight forms of endothelial cell growth factor. J Biol Chem. 1982 May 25;257(10):5333-6. PubMed PMID: 7068593.
2. Olander J (1980) In Vitro 6:209.
3. Folkman J, Haudenschild C. Angiogenesis in vitro. Nature. 1980 Dec 11;288(5791):551-6. PubMed PMID: 6160403.
4. Evans CH, DiPaolo JA. Equivalency of endothelial cell growth supplement to irradiated feeder cells in carcinogen-induced morphologic transformation of Syrian hamster embryo cells. J Natl Cancer Inst. 1982 Jan;68(1):127-31. PubMed PMID: 6275157.
5. Pintus C, Ransom JH, Evans CH. Endothelial cell growth supplement: a cell cloning factor that promotes the growth of monoclonal antibody producing hybridoma cells. J Immunol Methods. 1983 Jul 15;61(2):195-200. PubMed PMID: 6863945.
6. Maciag T (1979) PNAS 6:5674.
7. Thornton SC, Mueller SN, Levine EM. Human endothelial cells: use of heparin in cloning and long-term serial cultivation. Science. 1983 Nov 11;222(4624):623-5. PubMed PMID: 6635659.
8. Ransom JH. Endothelial cell growth supplements for promoting the growth of monoclonal antibody-producing hybridoma cells. Methods Enzymol. 1986;121:293-5. PubMed PMID: 3724467.
9. Schniedermann J, Rennecke M, Buttler K, Richter G, Städtler AM, Norgall S, et al. Mouse lung contains endothelial progenitors with high capacity to form blood and lymphatic vessels. BMC Cell Biol. 2010 Jul 1;11:50. doi: 10.1186/1471-2121-11-50. PubMed PMID: 20594323.

Protocols:

Thymidine Incorporation with HUVEC
- plate cells with a density at 5-7 x 10e3 cells/well in growth medium (EGM)
- incubate cells over night [if urgent, plate cells in the morning, change growth medium against basal medium (EBM) in the early afternoon]
- change EGF against EBM (for HUVEC: EBM +1-2% FCS)
- incubate 24 h
- change medium again and add factors (growth factors, inhibitors, etc.)
- incubate for 18 h
- add 10 µl 3H-Thymidine solution [0.025 mCi/ml] per well (=0.25 µCi)
- incubate another 6h at 37°C

- Washing steps: (250 µl/well)
PBS 1x
MeOH 2x 5 min
TCA 2x 10 min
H2O 1x
- lyse cells in 250 µl 0.3M NaOH per well
- transfer 2.5 ml ECO Plus into the appropriate scintillation vials
- transfer cell lysats into the scintillation vials
- count by liquid scintillation (ß-counter; Beckmann Instruments)