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Endothelial Cell Growth Factor (ECGF) **(Cell culture grade)**

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.

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 to cultivate 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.

Host species:	Bovine (BSE-free tested!)
Purification:	Crude extract
Buffer:	H ₂ O, w/o preservative
Formulation:	lyophilized

Reconstitution and Use: Endothelial cell growth factor is supplied as a sterile lyophilized powder containing 6 mg protein per vial. To obtain a stock solution reconstitute the contents of the vial in 2 ml of prewarmed (37 °C) sterile balanced salt solution. 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 6 mg ECGF are sufficient for 500 ml medium.**

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 10 µg/ml. As a growth supplement for use in monoclonal antibody production the optimum range is 25 to 100 µg/ml.

Species specificity: Bovine ECGF is effective on mouse, bovine and human cells.

Storage

Prior to reconstitution store vial at 2-8 °C. After reconstitution, the product may be stored as aliquots at -20 °C. It is recommended to store the reconstituted solution in aliquots at -20°C. **Repeated freezing and thawing should be avoided.**

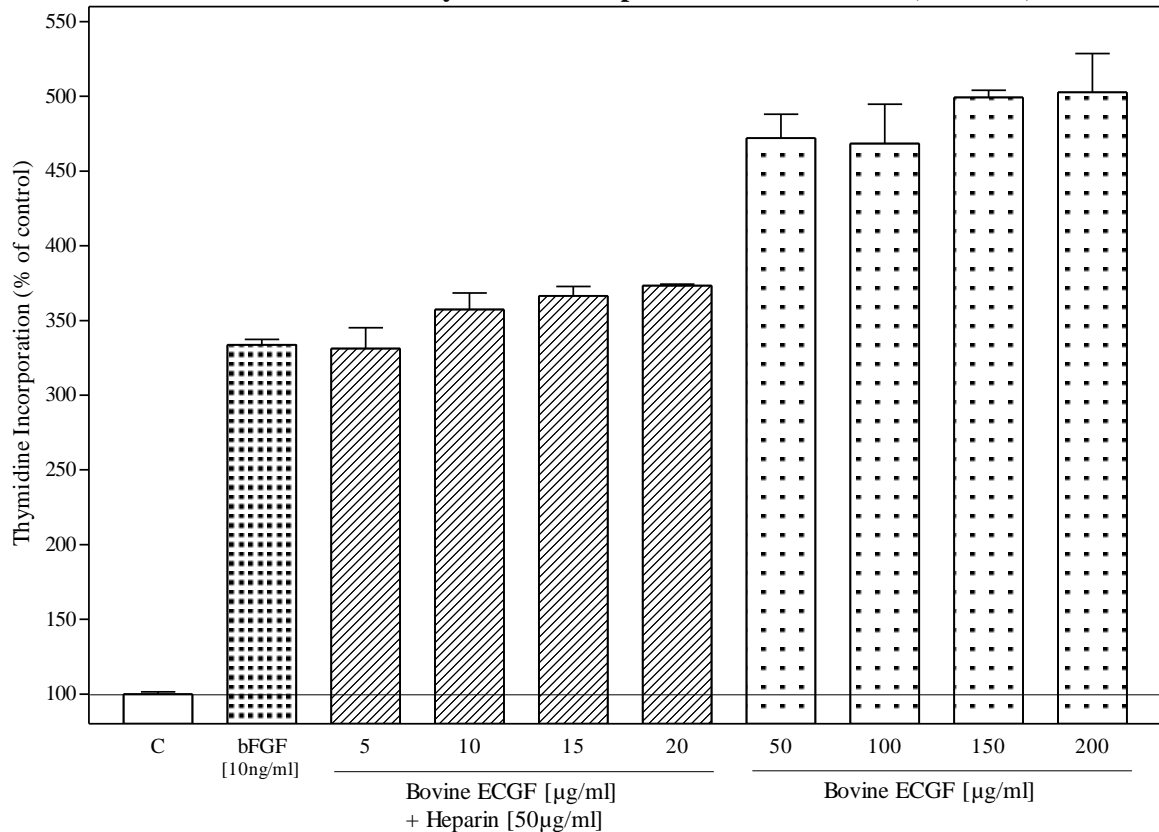
Usage: Bovine ECGF is offered for research use. Not for drug use. **Not for human use!**

Catalogue Number: 300-090-5

Size: 5x 6 mg

Literature: [Maciag T (1982) JBC 257:5333; Olander J (1980) In Vitro 6:209; Folkman J (1980) Nature 288:551; Evans CH (1982) JNCI 68:127; Pintus C (1983) J Immuno Meth 61:195; Maciag T (1979) PNAS 6:5674; Thornton SC (1983) Science 222:623; Ransom JH (1986) Methods Enzymol 121:293]

Bovine ECGF: Thymidine Incorporation with HUVEC (11.04.08)



Protocol:

- plate cells with a density at $5-7 \times 10^3$ 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 24h
- change medium again and add factors (growth factors, inhibitors, etc)
- incubate for 18h
- add $10\mu\text{l}$ ^3H -Thymidine solution [0.025mCi/ml] per well ($=0.25\mu\text{Ci}$)
- incubate another 6h at 37°C
- Washing steps: ($250\mu\text{l/well}$)

PBS	1x
MeOH	2x 5min
TCA	2x 10min
H ₂ O	1x
- lyse cells in $250\mu\text{l}$ 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 (β -counter; Beckmann Instruments)