

## TECHNICAL DATA SHEET

**Bovine ECGF**  
Bovine Endothelial Cell Growth Factor

PLEASE NOTE: ALWAYS CENTRIFUGE VIAL BEFORE OPENING

Size	Order #	Lot #	Expiry Date*
5x6 mg	W 1319919308	3608102230	March 2012

**Description**

Bovine 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 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.

- **Biological activity** See below
- **Source** *Natural, Bovine brain (BSE-free tested)*
- **Purity** Crude extract
- **Endotoxin level**  $\leq 0.1 \text{ ng}/\mu\text{g}$  ( $\leq 1 \text{ EU}/\mu\text{g}$ )
- **Stabilizer** None
- **Buffer**  $\text{H}_2\text{O}$ , w/o preservative
- **Physical state** Sterile filtered, lyophilized

**Biological Activity/ Working concentration**

Optimum concentration for human umbilical vein endothelial cells (HUVEC) range from 50-200  $\mu\text{g}/\text{ml}$ , optimal concentration with heparin (50  $\mu\text{g}/\text{ml}$ ) is about 10  $\mu\text{g}/\text{ml}$ . As a growth supplement for use in monoclonal antibody production the optimum range is 25 to 100  $\mu\text{g}/\text{ml}$ .

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

**Reconstitution**

Bovine ECGF 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.**

**Stability**

Bovine ECGF should be stored at 2-8°C. Upon reconstitution, the protein should be stored as aliquots at -20°C. It is recommended to store the reconstituted solution in aliquots at -20°C.

**Please avoid repeated freeze/thaw cycles.**

**Usage**

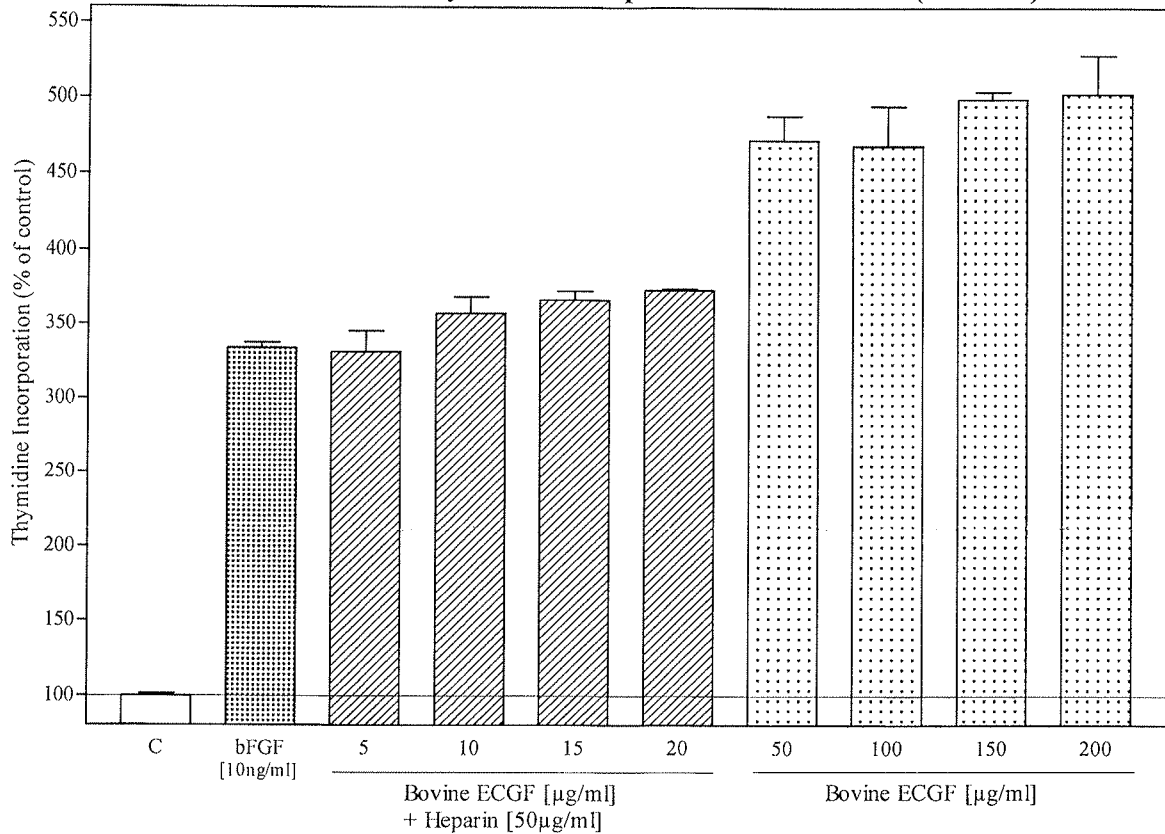
FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES

\*For lyophilized product if stored as indicated. Please contact our technical support if the product has expired.

Biochrom AG, Leonorenstr. 2-6, 12247 Berlin  
Phone: +49 30 77 99 06-92  
Fax: +49 30 771 00 12  
Email: [info@biochrom.de](mailto:info@biochrom.de)  
<http://www.biochrom.de>

### 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  $10\mu\text{l}$   $^3\text{H}$ -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 <sub>2</sub> 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 ( $\beta$ -counter; Beckmann Instruments)