



RECOMBINANT RAT LEUKEMIA INHIBITORY FACTOR (rtLIF)

CATALOG NUMBER: LIF3005

LOT NUMBER:

QUANTITY: 5 µg

CONCENTRATION: 10 µg/mL

DESCRIPTION: Leukemia Inhibitory Factor (LIF) is a lymphoid factor which promotes long-term maintenance of embryonic stem cells by suppressing spontaneous differentiation. LIF has a number of other activities including cholinergic neuron differentiation, control of stem cell pluripotency, bone and fat metabolism, mitogenesis of certain factor dependent cell lines and promotion of megakaryocyte production *in vivo*.

Rat LIF (rtLIF) is a 19.9 kDa protein containing 183 amino acid residues that exhibits 91% amino acid sequence identity with murine LIF¹. Analysis of the secondary structure of rat and murine LIF indicates a difference in the two dimensional structure of both proteins¹. Studies have shown that rtLIF is more effective in maintaining the undifferentiated phenotype of rat ES cells than similar concentrations of murine LIF¹.

SOURCE: rtLIF is expressed in *E. coli* as a fusion protein with GST using the pGEX expression system, cleaved from GST moiety with thrombin and purified by ion-exchange chromatography.

PURITY: Greater than 85% by analytical HPLC and SDS-PAGE. Endotoxin level is less than 0.1 ng per µg of LIF. Tested negative in both aseptic and mycoplasma tests.

ACTIVITY: The activity of rat LIF is determined by the ability to induce differentiation of M1 myeloid leukemic cells. The minimum detectable concentration of rat LIF in this assay is 0.5 ng/mL. The specific activity is $\geq 1 \times 10^8$ units/mg, where 50 units is defined as the amount of rat LIF required to induce differentiation in 50% of the M1 colonies in 1 mL agar cultures.

PRESENTATION: Liquid in 50mM sodium phosphate/1mM DTT / 10% glycerol / 250mM NaCl, pH 7.4 and 0.02% Tween 20. No preservatives added.

STORAGE/HANDLING: Rat LIF is shipped on dry ice. Maintain at -20°C until expiration date. Further dilutions should be made into buffer or medium to which protein (e.g., 1% BSA) or Tween 20 has been added and stored at -20°C. Freeze thawing is not recommended.

REFERENCES:

1. Takahama Y. *et. al.* (1998). "Molecular cloning and functional analysis of cDNA encoding a rat leukemia inhibitory factor: towards generation of pluripotent rat embryonic stem cells." *Oncogene* 16: 3189-3196.



RECOMMENDED PROTOCOL

M1 Bioassay

1. The M1 bioassay is performed using *in vitro* semi-solid agar cultures, which contain approximately 100 cells in 1 mL volumes of DME containing 20 % FCS in 0.3% agar.
2. Add 100 μ L of sample or rtLIF (10^4 units/mL in 5% FCS in isotonic saline) in two-fold serial dilutions in duplicate to 35 mm petri dishes.
3. Add 100 μ L of 5% FCS in isotonic saline to two control slides.
4. Incubate at 37°C in fully humidified atmosphere of 10% CO₂ in air for 7 days.
5. Score the number of colonies that show differentiation (note: 50 units is defined as the amount of activity which results in 50% of the colonies being differentiated).

Visit www.esgro-lif.com for additional information

Manufactured by Millipore. Rat LIF is not available for resale.

For research use only; not for use as a diagnostic.