

# rec. human Leukemia Inhibitory Factor

## rHu LIF

Product	Cat#	Package size
rec. human Leukemia Inhibitory Factor - rHu LIF	C4340.0005	5µg
rec. human Leukemia Inhibitory Factor - rHu LIF	C4340.0025	25µg
rec. human Leukemia Inhibitory Factor - rHu LIF	C4340.1000	1mg

**Synonyms:** CDF, HILDA, D-FACTOR, Differentiation- stimulating factor, Melanoma-derived LPL inhibitor, MLPLI, Emfilermin, Leukemia inhibitory factor, LIF, DIA.

### Product description

Leukemia Inhibitory Factor also called LIF is a lymphoid factor that promotes long-term maintenance of embryonic stem cells by suppressing spontaneous differentiation. Leukemia Inhibitory Factor has several functions such as cholinergic neuron differentiation, control of stem cell pluripotency, bone and fat metabolism, mitogenesis of factor dependent cell lines and promotion of megakaryocyte production in vivo. Human and mouse LIF exhibit a 78% identity in its amino acid sequence.

Recombinant human Leukemia Inhibitory Factor (rHu LIF) is produced in *E. Coli* as a non-glycosylated polypeptide of 180 amino acids and a total molecular mass of 19.7 kDa.

**Source:** *E. coli*

### Biological activity

The ED50 was determined by the M1 cell differentiation assay is <0.01ng/mL, corresponding to a specific activity of 100,000,000IU/mg.

**Purity:** >98% determined by SDS-PAGE and RP-HPLC

### Amino acid sequence:

SPLPITPVNA TCAIRHPCHN NLMNQIRSQL AQLNGSANAL FILYYTAQGE PFPNLDKLC GPNVTDFFPF HANGTEKAKL VELYRIVVYL GTSLGNITRD QKILNPSALS LHSKLNATAD ILRGLLSNVL CRLCSKYHVG HVDVTYGPDT SGKDVFKKK LGCQLLGKYYK QIIAVLAQAF.

**Formulation:** Leukemia Inhibitory Factor (LIF) was lyophilized from a concentrated (1mg/mL) sterile solution containing 1X PBS pH7.4.

### Stability

The lyophilized protein is stable for a few weeks at room temperature, but best stored at -20°C.

Reconstituted LIF should be stored in working aliquots at -20°C.

Reconstituted LIF can be stored for 2-7 days at 4°C.

**Please prevent from repeated freeze-thaw cycles!**

### Reconstitution

We recommend a quick spin followed by reconstitution in water to a concentration of not less than 100µg/mL which can then be further diluted to other aqueous solutions.

### Usage

This product is for research/laboratory usage only. It may not be used as drug, agricultural or pesticidal product, food additive or household chemical.

## Application

Rec. human LIF might be used as a reagent for the in vitro maintenance of the pluripotential phenotype of murine and human ES cells.

## Protocol

In D3 and MBL-1 pluripotential ES cell cultures it is found that 1000 units of rec. human LIF per 1.0mL of tissue culture media is required to maintain ES cells with a stem cell phenotype. Similar concentrations of huLIF have also been used for germ line transmission of genetically altered ES cells

At the recommended concentration  $10^7$  units of huLIF is sufficient for 10L of tissue culture media and  $10^6$  units are sufficient for 1L of cell culture media.

### Mouse cell cultivation with LIF

- Transfer mouse cells to a Gelatine coated (0.1%) dish with pre-warmed mouse cell maintenance medium.
- Culture the cells at +37° C and 5% CO<sub>2</sub> for 3 to 5 days or until just before the cell colonies contact each other.
- Replace the mouse cell maintenance medium daily.

### Mouse cell splitting

- Aspirate the medium from the tissue culture dish
- Gently rinse dish with PBS
- Add Trypsin (0.05% Trypsin, 0.1% EDTA) for 5 minutes. Keep the time of Trypsin exposure to a minimum. Use FBS to inactivate Trypsin.
- When colonies are separated into single cells transfer cells into a new tube.
- Centrifuge at maximum 340xg for approx. 3 minutes to pellet the cells.
- Discard the supernatant.
- Resuspend the cells in the desired volume of mouse cell maintenance medium, transfer to new dishes and incubate.
- Mouse cells generally should not be split at ratios lower than 1:4 or higher than 1:10. Cells should be split when they reach ca. 80% confluency.

Table 1: Mouse Cell Maintenance Medium

Component	Vol. added for 100mL	Final conc.
FBS, pre-tested on ES cells	15.0mL	15%
Non-essential amino acids (100X) (C4285)	1.0mL	1X
Leukemia Inhibitory Factor (LIF) (C4340)	1µg	$10^5$ units / 100mL medium
Sodium Pyruvate, 100mM (C4214)	1.0mL	1mM
2-mercaptoethanol		100µM
L-Glutamine, 200mM (C4281)	1.0mL	2mM
DMEM without glutamine (C4302)	To final vol. of 100mL	

\* For preparation of 1L culture medium please add 10µg recombinant human LIF

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