Recombinant Human Leukemia Inhibitory Factor (LIF)

(Cat. No.: C017)

Background:

Leukemia Inhibitory Factor (LIF) is a ly mphoid 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. Human and murine mature LIF exhibit a 78% sequence identity at the amino acid control. Human LIF is equally active on both human and mouse cells. Murine LIF is approximately 1000 fold less active on human cells, than hLIF.

Description:

Recombinant Human LIF produced in *E. coli* is a single, non-glycosylated, polypeptide chain containing 180 amino acids and having a molecular mass of 19717 Dalton.

Quality Control:

Biological activity: The ED50 was determined by the M1 cell differentiation assay is < 0.01 ng/ml, corresponding to a specific activity of 1.0×10^8 IU/mg.

Purity: Greater than 95% as determined by

(a) Analysis by RP-HPLC.

(b) Anion-exchange FPLC.

(c) Analysis by reducing and non-reducing SDS-PAGE Silver Stained gel..

Amino-Acid Sequence: The sequence of the first five N-terminal amino acids was determined and was found to be Ser-Pro-Leu-Pro-Ile.

Endotoxin: Less than 0.1ng/μg (1IEU/μg) of LIF.

Formulation: LIF was lyophilized after extensive dialysis against PBS.

Storage: Lyophilized rHuLIF although stable at room temperature for 3 weeks, should be stored desiccated below -18°C. Upon reconstitution rHuLIF should be stored at 4°C between 2-7 days and for future use below -18°C. For long-term storage it is recommended to add a carrier protein (0.1% HSA or BSA).

Please avoid freeze-thaw cycles.

Reconstitution: We recommend a quick spin followed by reconstitution in water to a concentration of 0.1-1.0mg/ml. It is recommended that further dilutions be made into buffer or medium to which protein (e.g., 1% BSA) or Tween 20 has been added. This solution can then be stored at 4°C for 1 week or -20°C for future use.

Example: Using SinoBio's LIF in W4 ES Cell Culture

Embryonic Stem Cell Culture Assay:

Mouse embryonic stem Cells (W4) are cultured in media containing SinoBio's recombinant human Leukemia Inhibitory Factor (LIF) [Cat. C017] at the concentration of 1000 IU/mL. After 10 passages (25 days), the ES cell colonies show normal morphology (Figure. 1) and the karyotype results of these ES cells indicate there are 80% ES cells contain the normal modal chromosome number of 40 (Figure. 2).

Same concentration of mLIF from CHEMICON has also been assessed side by side in this assay, and show similar results.

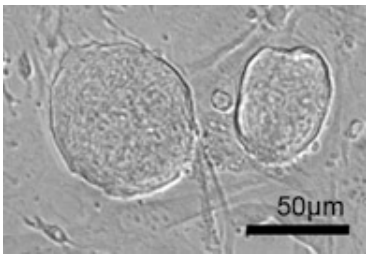

Figure. 1

W4 ES cells cultured for 10 passages, 25 days, in media containing SinoBio's Leukemia Inhibitory Factor (LIF) [Cat. C017]. A concentration of 1000 IU/mL is used for inhibition of differentiation.

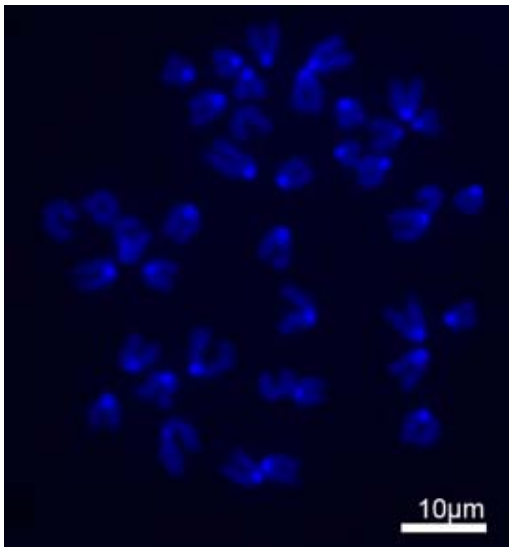

Figure. 2

A representative karyotype result of the long-term (10 passages, 25 days) cultured W4 ES cells. The number of chromosome (stained with DAPI) is 40.

Test for Germ-line Transmission of the Long-term Cultured ES Cells:

After 10 passages (25 days), the W4 ES cells maintained in media containing SinoBio's Leukemia Inhibitory Factor (LIF) [Cat. C017] are microinjected into C57/BL6 blastocysts, they can still give rise to male chimeras with significant ES contribution (>95%, as determined by an agouti coat color). Two male chimeras are crossed with female C57BL/6J, all the offspring (n=26, 4 litters) carry the agouti coat color gene driven from the W4 ES cells. This result shows the successful germ-line transmission of the long-term cultured ES cells.