



MACS® Cytokines

Mouse LIF for reliable ESC culture

Background information

Leukemia inhibitory factor (LIF) is a pleiotropic cytokine, which is critically involved in embryonic development and blastocyst implantation. LIF belongs to the interleukin-6 family and functions through the gp130 activation of STAT3. LIF affects hematopoiesis, neural development, bone and energy metabolism, and inflammation. In mice, LIF is a key factor that prevents differentiation of mouse embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Thus, LIF is essential for the maintenance of self-renewal and pluripotency in conventional mouse ESC and iPSC cultures.

Product information

Source: *E. coli*

Sequence: 180 amino acid polypeptide (without tag or *E. coli* derived methionine)

SPLPITPVNATCAIRHPCHGNLMNQIKNQLAQLNGSAN
ALFISYYTAQGEFFPNNVEKLCAPNMTDFPSFHNGTE
KTKLVELYRMVAYLSASLTNITRDQKVLNPTAVSLQVK
LNATIDVMRGLLSNVLCRLCNKYRVGHVDVPPVDPHSD
KEAFQRKKLGCQLLGTYKQVISVVQAF

Purity: >97% as determined by SDS-PAGE (fig. 1)

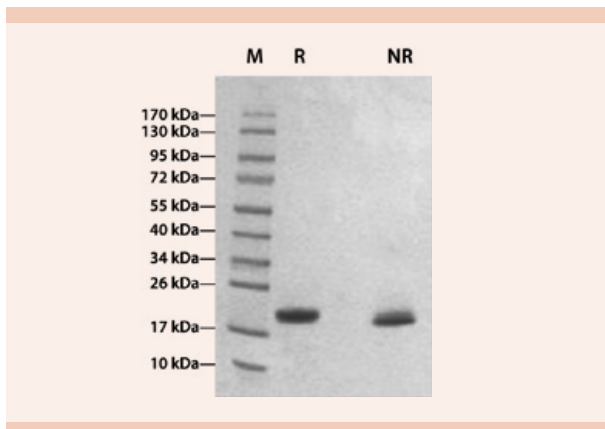


Figure 1: SDS-PAGE of pure Mouse LIF under reducing (R) and non-reducing (NR) conditions; M: marker.

Biological activity: Determined by a proliferation inhibition assay using mouse M1 myeloid leukemic cells according to Rose and Bruce¹. The ED₅₀ is <0.5 ng/mL (fig. 2) and corresponds to a specific activity of >2×10⁶ units/mg. Note that one unit of MACS® Mouse LIF corresponds to 50 units of the Millipore product.

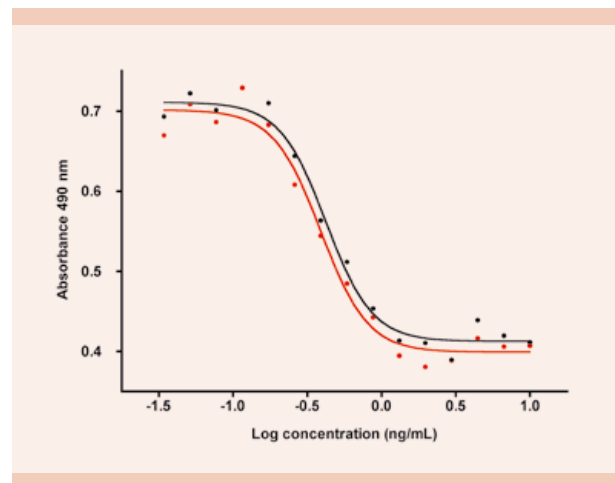


Figure 2: Mouse LIF activity assay. Activity of MACS Mouse LIF, premium grade (red line) was compared to another commercially available product (black line) with fully equivalent results.

Endotoxin level: low endotoxin (<0.1 EU/μg cytokine) as determined by *Limulus* amoebocyte lysate (LAL) assay.

Applications

Characterization of mouse ESCs after feeder-free cultivation in the presence of Mouse LIF

Mouse ESCs were cultured for six days in ES medium supplemented with 20 U/mL Mouse LIF. Cells were fixed two days after splitting the culture. Light-microscopic (fig. 3A) or immunofluorescence (fig. 3B) images confirm the typical morphology and Oct4 expression.

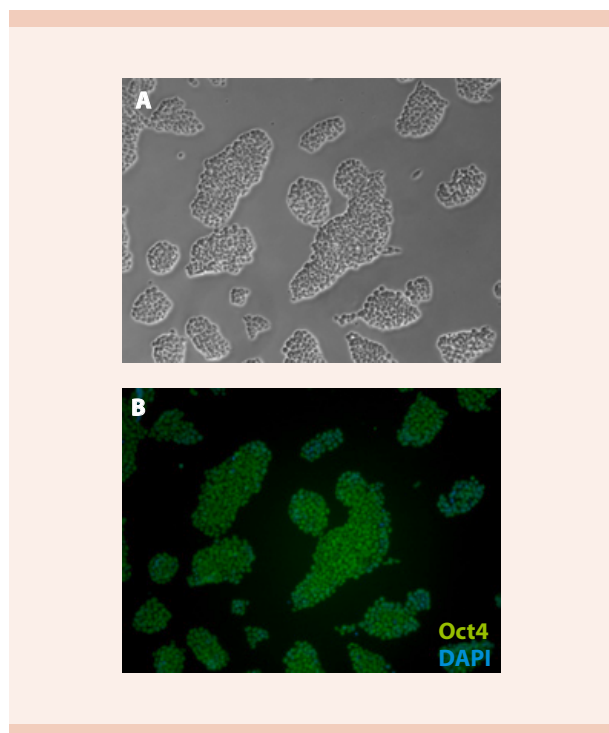


Figure 3: Light-microscopic and immunofluorescence images of ESCs cultured in the presence of Mouse LIF.

Flow cytometric analysis of further established pluripotency markers expressed in ESCs

ESCs were cultured under feeder-free conditions in the presence of MACS Mouse LIF (20 U/mL) or another commercially available LIF (MP, 1000 U/mL). Cells were

labeled with CD15-APC, CD326 (EpCAM)-APC, or CD324 (E-cadherin)-APC antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer (fig. 4).

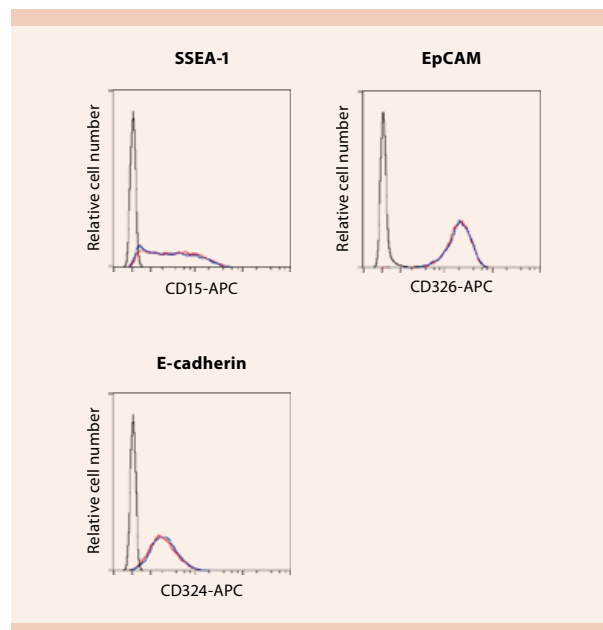


Figure 4: Flow cytometric analysis of ESCs cultured in the presence of MACS Mouse LIF or another commercially available LIF. MACS Mouse LIF: blue; MP LIF: red; isotype control: black.

Conclusion

MACS Mouse LIF effectively maintains pluripotency of ESCs and thus represents a reliable supplement for routine ESC cultivation. A minimum concentration of 10 ng/mL or 20 U/mL is required to support mouse ESCs in an undifferentiated state. Certificates of analysis with lot-specific biological activities are available for premium-grade MACS Cytokines upon request for exact unit dosing. This renders routine activity assays unnecessary and ensures consistent results.

Reference

1. Rose, T.M. and Bruce, A.G. (1991) Proc. Natl. Acad. Sci. USA 88: 8641–8645.

MACS Products

Product name	Product description	Source	Packing unit	Order no.
Mouse LIF	Recombinant mouse leukemia inhibitory factor	<i>E. coli</i>	10 µg	130-095-772
		<i>E. coli</i>	25 µg	130-095-775
	<i>Biological activity:</i> ≥ 1×10 ⁶ U/mg; inhibition of M1 cells			
Mouse LIF, premium grade	Recombinant mouse leukemia inhibitory factor	<i>E. coli</i>	10 µg	130-095-777
		<i>E. coli</i>	25 µg	130-095-778
	<i>Biological activity:</i> ≥ 2×10 ⁶ U/mg; inhibition of M1 cells	<i>E. coli</i>	100 µg	130-095-779



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