

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# **Product Information**

# Anti-Leukemia Inhibitory Factor produced in goat, affinity isolated antibody

Catalog Number L1044

# **Product Description**

Anti-Leukemia Inhibitory Factor (rmLIF) is produced in goats immunized with purified, *E. coli*-derived, recombinant mouse leukemia inhibitory factor (GeneID 16878). The antibody is purified by mouse LIF affinity chromatography.

Anti-Leukemia Inhibitory Factor recognizes mouse leukemia inhibitory factor. Applications include immunoblotting, and neutralization of rmLIF. Based on western blot results (non-reducing conditions), this antibody shows less than 5% cross-reactivity with rhLIF.

Leukemia inhibitory factor is a multifunctional glycoprotein that induces macrophage differentiation and suppresses the proliferation of the murine M1 myeloid cell line.<sup>1</sup>

### Reagent

Supplied lyophilized from a  $0.2 \, \mu m$  filtered solution of phosphate buffered saline with 5% trehalose.

# **Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### **Preparation Instructions**

To one vial of lyophilized powder, add 1 mL of 0.2  $\mu$ m filtered PBS to produce a 0.1 mg/mL stock solution. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

#### Storage/Stability

Prior to reconstitution, store at -20 °C. The reconstituted product may be stored at 2-8 °C for up to one month. For extended storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended.

#### **Product Profile**

Immunoblotting: a working concentration of 0.1-0.2  $\mu$ g/mL is recommended. The detection limit for recombinant mouse LIF is ~2.0 ng/lane under non-reducing and reducing conditions.

Neutralization: To measure the ability of the antibody to neutralize the bioactivity of rmLIF on mouse DA-1a cells, rmLIF was incubated with various concentrations of the antibody for 1 hour at 37° C in a 96 well microplate. Following this preincubation period, DA-1a cells were added. The assay mixture in a total volume of 100 uL, containing antibody at concentrations ranging from 0.0001-1.0 µg/mL, rmLIF at 0.5 ng/mL and cells at 5 x 10<sup>4</sup> cells/mL, was incubated at 37° C for 68 hours in a humidified CO<sub>2</sub> incubator. The pale yellow tetrazolium salt 3-(4.5 dimethylthiazol-2yl) - 2, 5 diphenyl tetrazodiumbromide (MTT), at 5 mg/mL in PBS, was added for the final four hours (25  $\mu$ L/well). The dark blue formazan crystals, from the reduction of MTT by various dehydrogenases in the mitochondria, are presolubilized by the addition (100 µL/well) of the solubilization solution (50% v/v dimethyl formamide, 20% (w/v) SDS, pH 4.7). After an overnight incubation to ensure that the crystals have completely dissolved. the optical density of each well was measured in a microplate reader set at 540 nm. For background noise subtraction, the second wavelength was set at 690 nm.

The Neutralization  $\mathsf{Dose}_{50}$  (ND<sub>50</sub>) for this antibody is defined as that concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

**Note**: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilutions by titration.

Endotoxin: < 0.1 EU/ $\mu g$  antibody as determined by the LAL method.

## References

- 1. Gearing, D., et al., *EMBO J.*, **6**, 3995 (1987).
- 2. Moreau, F.J., et al., Nature, 336, 690 (1988).

RC,SC,PHC 07/11-1