Antibody Datasheet

Product name
Human Granulocyte Macrophage Colony-Stimulating Factor antibody (1-9F)

Product description
Human monoclonal antibody to hGM-CSF

Catalog Number
EVHM0102-100

Source
Human (recombinant production in CHO-K1)

Clonality and Clone name
Monoclonal, 1-9F

Isotype
IgG1 Lambda

Form Supplied and Size
Liquid, 100 μg

Concentration and storage buffer
1 mg/mL in Phosphate buffer saline pH 7.4 (containing no preservative)

Storage
Antibody can be kept at 4°C for up to 1 month and should be kept at -20°C or below for long-term storage. To avoid repeated freeze thaw cycles, antibody should be aliquoted before frozen.

Purification
Purified by protein A chromatography. The purity is greater than 95% by SDS-PAGE.

Antigen for Screening
Recombinant hGM-CSF produced by *E.coli*

Epitope
Epitope has not been determined.

Applications
ELISA, Neutralization assay. Other applications have not been tested

Limitations
This product is to be used for research purposes only.

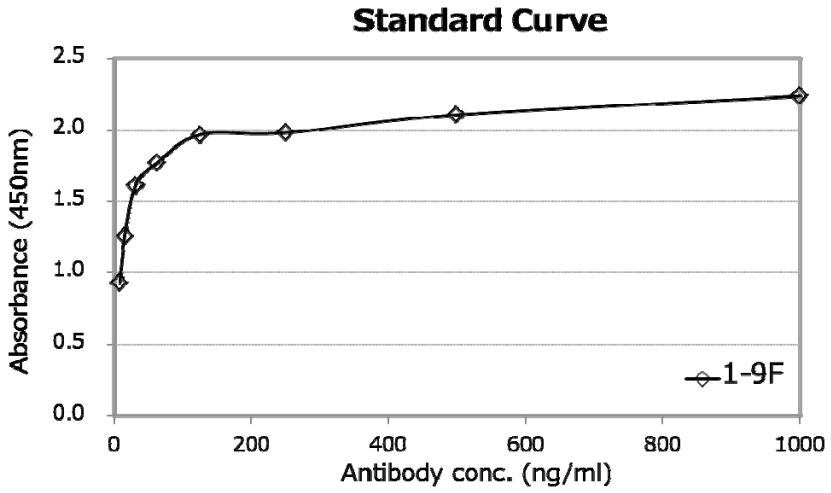
Background information
Granulocyte/Macrophage colony-stimulating factor (GM-CSF) is a hematopoietic growth factor that stimulates the development of neutrophils and macrophages and induces the proliferation and development of erythroid, megakaryocyte and eosinophil progenitors. GM-CSF is produced by endothelial cells, fibroblasts, activated T cells, NK cells and macrophages. The antibody reacts with human GM-CSF and can neutralize the bioactivity of natural or recombinant GM-CSF.

Immunogen and Recombinant Production Host
This antibody was generated from a healthy individual by a method based on Epstein-Barr virus transformation of peripheral blood mononuclear cells followed by the isolation of antibody-producing cells. The antibody reactivity for the target antigen was screened by enzyme-linked immunosorbent assay (ELISA) using recombinant hGM-CSF. The antibody genes were cloned from the antibody-producing cells and introduced into CHO-K1 cells for antibody production.

Application Note
Recommended Starting Dilutions:
For ELISA: Use at 1:500 – 1:4000
Not yet tested in other applications.
The optimal working dilution should be determined experimentally by the end user.

Neutralization assay
GM-CSF requiring TF-1 proliferation (see Procedure, “Neutralizing assay”)
The 50% and 90% inhibitory dose (IC50 and IC90) were calculated as the concentration of anti-hGM-CSF (1-9F, IC50 ≥ 8.6 ng/ml and IC90 ≥ 183.2 ng/ml) within the range of inhibition curve. Neutralization effect of 1-9F was observed even at low concentration, however 1-9F could not completely inhibit TF-1 proliferation even at 8 μg/ml.

ELISA Results



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NEUTRALIZATION ASSAY USING TF-1 CELLS

INTRODUCTION

TF-1 cells are derived from erythroleukemia patients. The cells require hGM-CSF or IL-3 cytokine for its proliferation. Cells are maintained by suspension culture.

PROCEDURE

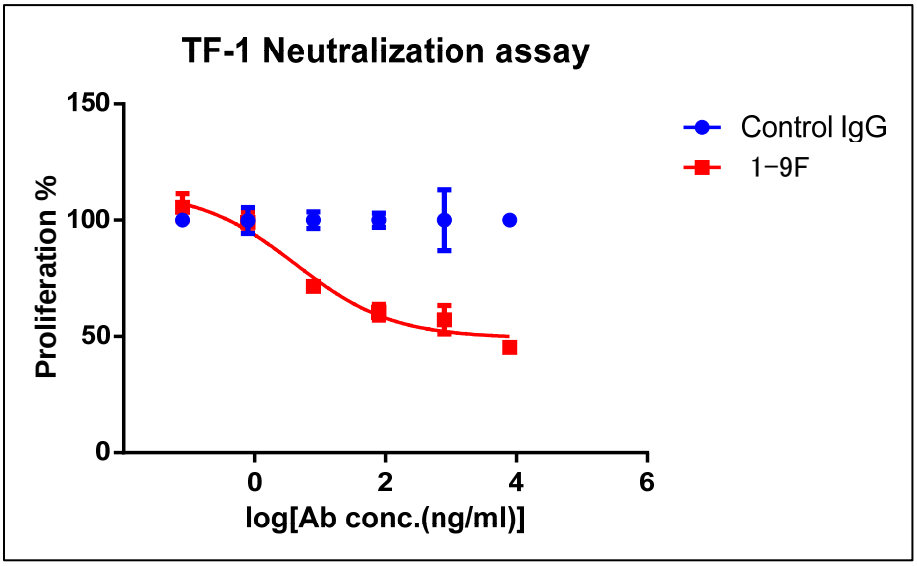
CELL MAINTENANCE AND PREPARATION

TF-1 cells are routinely maintained with 10% FBS-RPMI medium containing hGM-CSF (f.c. 1 ng/ml). Human GM-CSF should be supplied every other day. Final passage before neutralizing assay, medium is changed into macrophage SFM with same concentration of human GM-CSF.

Seed cells at 5×10^4 cells/mL or higher in Growth Medium.
Split cells every 2-3 days and reseed in fresh Growth Medium.

TF-1 NEUTRALIZATION ASSAY

1. Ahead of neutralization assay, wash TF-1 cells with Macrophage SFM without hGM-CSF to eliminate endogenous hGM-CSF that is present in the old medium.
2. Incubate for 3 hrs at 37 °C, 5% CO_2.
3. Prepare antibody 4-fold serial dilution solutions (from 32 ug/ml~) and 2 ng/ml of GM-CSF solution.
4. Use equal volume of antibody and antigen solutions for mixing.
5. Pre-incubate mixture for 1hr at 37 °C.
6. TF-1 cell-counting: collect cells by centrifugation (1,200 rpm).
7. Re-suspend cells with Macrophage SFM and adjust 2.4 x 10^5 cell/ml.
8. Add equal volume of washed cells into each well.
9. Keep plates at 37 °C, 5% CO_2 for 40 hrs.
10. Perform WST-1 cell proliferation assay using a commercial kit.



COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
URL: http://www.cosmobio.co.jp e-mail:export@cosmobio.co.jp
[Outside Japan] Phone : +81-3-5632-9617 [国内連絡先] Phone : +81-3-5632-9610
FAX : +81-3-5632-9618 FAX : +81-3-5632-9619