# IS Giant Cell Tumor Conditioned Medium (IS GCT-CM)

| Catalog # | Product | Size |
|---|---|---|
| 91006 | IS Giant Cell Tumor Conditioned Medium (IS GCT-CM) | 50 mL liquid |

## Intended Use

FUJIFILM Irvine Scientific's Giant Cell Tumor Conditioned Medium (IS GCT-CM) can be used as a mitogen or growth factor for cytogenetic cultures of human bone marrow and peripheral blood cells. In addition, IS GCT-CM is intended to increase the efficiency of HIV infection in monocytes and macrophages and enhance the production and growth of human and heterohybridomas.

## Product Description

IS GCT-CM is prepared from a cultured giant cell tumor line derived from a human malignant fibrous histiocytoma (1) and constitutively produces a variety of growth factors.

IS GCT-CM is an excellent supplement for colony stimulating activity of hematopoietic progenitor cells due to the complementary activities of granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), and human erythroid enhancing activity (1,2). The GCT cell line also produces other macrophage-like cell factors such as interleukin-1 (IL-1), interleukin-6 (IL-6), a plasminogen activator, collagenase, and prostaglandin E.

## Quality Assurance

Do not use any medium that is not reddish orange in color, or that shows evidence of particulate matter or cloudiness. Discard the product in accordance with applicable regulations.

Sterile filtration is performed by means of a capsule filter with a pore size of 0.2 µm.

Sterility testing is performed according to USP XXIII guidelines (sampling by filtration checking for bacterial growth in Fluid thioglycollate medium, Trypticase soy broth and Sabouraud's broth).

The media is tested for presence of mycoplasma in growth agar and broth.

Endotoxin quantitation is determined by the Limulus Amebocyte Lysate Chromogenic Assay: <1 ng/mL.

Biological activity: CHANG Medium BMC test

## Quality Control

Quality Control of GCT-CM includes: pH, osmolality and bioburden

The Giant Cell Tumor line used for the production of this product tested negative for hepatitis B surface antigen, HIV, and mycoplasma.

## Storage Instructions and Stability

IS GCT-CM is stable when stored unopened at -10°C for up to two (2) years from the date of manufacture. See bottle label for specific expiration date. IS GCT-CM may be refrozen two (2) times without appreciable loss of activity, however it is recommended that the product be aliquoted into single use volumes so that multiple freeze-thaw cycles will not be required.

## Suggestions for Use

*Growth of Hematopoietic Cells*

IS GCT-CM is a potent source of the colony stimulating factors necessary for growth of hematopoietic progenitor cells from human, mouse or rabbit bone marrow or peripheral blood (1,4). GM-CSF in combination with G-CSF or M-CSF (all present in IS GCT-CM) is required to obtain the maximum number of granulocyte and macrophage colonies. Serum-free cultures of human hematopoietic progenitors form bone marrow cells in soft agar or methylcellulose using a combination of GM-CSF with G-CSF or M-CSF with G-CSF, this as evidenced by increased colony size and number of colonies compared to those obtained with each of the factors alone (6).

Suggested concentration for use*:*

IS GCT-CM should be titrated to find the optimum concentration for each application and set of conditions. Concentrations of GCT-CM between 5 and 20% (v/v) have yielded growth of a variety of bone marrow (1) or peripheral blood cells (4) in semi-solid medium. The number of colony forming cells (CFC) obtained from human bone marrow progenitor cells is typically determined from a culture of 1 - $2x10^6$ bone marrow cells/mL.

The number of CFC obtained from rabbit or mouse bone marrow cells is typically determined from cultures of $4x10^4$ – $1x10^5$ bone marrow cells/mL.

*Growth of Leukemia Cells*

IS GCT-CM is a potent source of colony stimulating factors for growth of myeloid leukemia cells isolated from peripheral blood of patients with chronic myelogenous leukemia (7). GM-CSF and G-CSF (contained within GCT-CM) can promote the growth of larger colonies from peripheral blood of patients with acute myeloblastic leukemia than either factor alone (8). Similarly, GCT-CM allowed the proliferation of bone marrow cells from a patient with acute myeloid leukemia (9) in liquid culture or colonial growth in semi-solid medium. These cells could be distinguished from the proliferating cells that were obtained when the patient was in remission after chemotherapy.

*Suggested concentration for use:*

IS GCT-CM should be titrated to find the optimum concentration for each application and set of conditions. Typical concentrations of GCT-CM for maximum stimulation of leukemia cells range between 5 and 10% (v/v) in growth medium (7).

*Cytogenetic Analysis of Bone Marrow Cells*

Cytogenetic analyses of bone marrow cells can be enhanced by the addition of IS GCT-CM to improve the length and morphology of chromosomes and increase the mitotic index of specimens obtained that have normal karyotypes, or abnormal karyotypes in patients diagnosed with lymphoid leukemia, preleukemia, or chronic myelogenous leukemia (10).

*Suggested concentration for use:*

IS GCT-CM should be titered to find the optimum concentration for each application and set of conditions. A 10% (v/v) concentration of GCT-CM used during the 24-hour culturing of bone marrow specimens before slide preparation was shown to enhance the cytogenetic analyses (10).

*HIV Isolation and Recovery*

IS GCT-CM is a good adjunct for human immunodeficiency virus (HIV) culture in monocytes and macrophages. M-CSF (contained within GCT-CM) allows normal blood-derived monocytes/macrophages to proliferate in culture. These cells are susceptible target cells for infection and isolation of HIV from peripheral blood monocytes of patients with AIDS and ARC (11). The frequency of obtaining HIV infected macrophage cultures using the co-culture method is improved by the addition of GCT-CM (55% isolation rate) versus recombinant M-CSF alone (21% isolation rate) (12). This suggests that the combination of GM-CSF and M-CSF also independently improves the yield of HIV antigen obtained from infected macrophage cultures (13), which may be due to increased proliferation of the cells.

*Suggested concentration for use:*

IS GCT-CM should be titrated to find the optimum concentration for each application and set of conditions. HIV recovery and isolation from asymptomatic seropositive patients may be enhanced by the co-culture of patient peripheral blood cells with monocyte-derived macrophages (11). The target monocytes are cultured in medium containing 10% (v/v) human serum and 10% (v/v) IS GCT-CM under non-adherent conditions. Patient peripheral blood cells are co-cultured for 48 hours and then removed. The monocytes are kept in culture and the supernatant fluids monitored for HIV p24 antigen (12).

*Production and Growth of Human Hybridomas*

IS GCT-CM contains human IL-6 which enhances the production of hybridomas made with human lymphocytes and human or human-mouse myelomas. IS GCT-CM can also be used for subcloning EBV-transformed cell lines.

*Suggested concentration for use:*

IS GCT-CM should be titered to find the optimum concentration for each application and set of conditions. A concentration of 5 to 10% (v/v) of IS GCT-CM added to standard culture medium enhances the growth of the hybridomas after fusion or after subcloning of either hybridomas or EBV-transformed lymphocytes.

*Instructions for Growth of Hematopoietic Cells from Murine Bone Marrow Cells*

Mice (BALB/c or other strain), 3-7 months old

Reagents:

- IS Giant Cell Tumor-Conditioned Medium (IS GCT-CM)
- cIMDM: Iscove's Modified Dulbecco's Medium containing 20% Fetal Bovine Serum (FBS, heat inactivated) and antibiotics (e.g. kanamycin sulfate)
- 3% Agar (e.g. Bacto-Agar or agarose) in water
- Crystal violet in 1% acetic acid
- INT (2-p-nitrophenyl-3-nitrophenyl-5-phenyltetrazolium chloride), 1 mg/mL in 0.85% NaCl and sterile filtered

## Directions for Use

1. Collect bone marrow cells from the femurs of the mice by aseptically removing the femurs from the mice and cutting off both ends of the bone.

2. Boil the 3% agar solution for 2 minutes and allow to cool to 45°C.

3. Count the bone marrow cells using a hemocytometer and crystal violet. Typically, $5x10^6$ cells are obtained from each femur.

4. Combine the appropriate amounts of IS GCT-CM, bone marrow cells, and medium to yield a final concentration between 5 and 20% (v/v) of IS GCT-CM and between $4 \times 10^4$ and $1 \times 10^5$ cells/mL after dilution with agar (refer to step 5). Keep the mixture at 37°C until use.

5. Add one part agar to nine parts of the cell mixture and immediately place the mixture into 96-well plates at 50 to 100µL per well, leaving the outer rows of wells empty. Fill the empty wells with sterile medium or water to humidify the plate.

6. Allow the agar to harden by leaving the plate at room temperature in a plastic box containing a wet paper towel (to prevent drying of the mixture) for 30 minutes.

7. Transfer the humidified box (the lid is loosely attached) to an incubator (37°C, 5% $CO_2$/air) and incubate for 7 days.

8. Add 50µL of the INT solution to each well of the 96-well plate. Return the plates to the incubator for another 18 to 24 hours.

9. Using a microscope, count the number of colonies in each well. The colonies are stained red by the INT for easier visualization. Adjust the microscope so that colonies below the agar surface can also be counted.

**Test several lots of serum to maximize the number of colonies obtained.

## References


1. DiPersio, J.F., Brennan, J.K., Lichtman, M.A., Speiser, B.L. Human Cell Lines that Elaborate Colony-Stimulating Activity for the Marrow Cells of Man and Other Species. Blood 1978; 51:507-519.

2. Erickson-Miller, C.L., Abboud, C.N., Brennan, J.K. Purification of GCT Cell-Derived Human Colony-Stimulating Factors. Experimental Hematology 1988; 16:184-189.

3. Das, S.K., Stanley, E.R., Guilbert, L.J., Forman, L.W. Description of a Colony-Stimulating Factor Subclass by a Specific Receptor on a Macrophage Cell Line. Journal of Cell Physiology 1980; 104:359-366.

4. Abboud, C.M., DiPersio, J.F., Brennan, J.K. Lichtman, M.A. Erythropoietic Enhancing Activity (EEA) Secreted by the Human Cell Line GCT. Journal of Supramolecular Structure 1980; 13:199-209.

5. Abboud, C.N. Brennan, J.K., Barlow, G.H., Lichtman, M.A. Hydrophobic Absorption Chromatography of Colony-Stimulating Activities and Erythriod Enhancing Activity from the Human Monocyte-Like Cell Line GCT. Blood 1981; 58:1148-1154.

6. Barlow, G.H., Brennan, J.K., Abboud, C.N., Francis, C.W., Lichtman, A.H., Lichtman, M.A. Secretion of Plasminogen Activator by the Human Macrophage-Like Cell Line, GCT: Separation from Colony-Stimulating and Erythropoiesis-Enhancing Activities. British Journal of Haemotology 1982; 52:645-655.

7. Abboud, C.N., Brennan, J.K., Lichtman, M.A., Nusbacher, J. Quantitation of Erythroid and Granulocytic Precursor Cells in Plateletpheresis Residues. Transfusion 1980; 20:9-16.

8. Sieff, C.A., Ekern, S.C, Nathan, D.G., Anderson, J.W. Combinations of Recombinant Colony-Stimulating Factors are Required for Optimal Hematopoietic Differentiation in Serum-Deprived Culture. Blood 1989; 73:688-693.

9. McNeice, I.K., Stewart, M.F., Deacon, D.M., Quesenberry, P.J. Synergistic Interactions Between Hematopoietic Growth Factors as Detected by in Vitro Mouse Bone Marrow Colony Stimulation. Experimental Hematology 1988; 16:383-388.

10. Griffin, J.D., Sullivan, R., Beveridge, R.P., Larcom, P., Schlossman, S.F. Induction of Proliferation of Purified Human Myeloid Progenitor Cells: A Rapid Assay for Granulocyte Colony-Stimulating Factors. Blood 1984; 63:904-911.

11. Vellenga, E., Young, D.C., Wanger, K., Wiper, D., Ostapovicz, D., Griggin, J.D. The Effects of GM-CSF and G-CSF in Promoting Growth of Clonogenic Cells in Acute Myeloblastic Leukemia. Blood 1987; 69:1771-1776.

12. Brennan, J.K., DiPersio, J.F., Abboud, C.N., Lichtman, M.A. The Exceptional Responsiveness of Certain Human Myeloid Leukemia Cells to Colony-Stimulating Activity. Blood 1979; 54:1230-1239.

13. Morgan, S., et. al., Qualitative and quantitative enhancement of bone marrow cytogenetics by addition of giant cell tumor-conditioned medium. Karyogram 1987; 13: 39-40.

# Technical Support

## CONTACT US

For more information or assistance contact Customer Service at:

- Email: tmrequest@irvinesci.com
- Direct line: +1 800 577 6097

## WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- Safety Data Sheets (SDS)
- Certificate of Analysis (CoA) (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

**FUJIFILM Irvine Scientific, Inc.**
2511 Daimler Street, Santa Ana, California 92705-5588 USA
Telephone: 1 949 261 7800 • 1 800 437 5706
Fax: 1 949 261 6522 • www.irvinesci.com