**IS GIANT CELL TUMOR CONDITIONED MEDIUM (GCT-CM)**

**Catalog Nos. 91006-50 mL**

**INTENDED USE**

The Giant Cell Tumor Conditioned Medium (IS GCT-CM) can be used as a Mitogen or growth factor for any Mitogen-free media for human bone marrow and peripheral blood cell culturing in the Cytogenetics industry. In addition, the 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 Giant Cell Tumor-Conditioned Medium (GCT-CM) is prepared from a cultured giant cell tumor line. The GCT cell line was derived from a human malignant fibrous histiocytoma (1) and constitutively produces a variety of growth factors.

IS GCT-CM is an excellent source of colony stimulating activity for 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.

**STABILITY AND STORAGE**

IS Giant Cell Tumor-Conditioned Medium is stable at -10°C for at least two years form date of manufacture and may be frozen and thawed at least five times without appreciable loss of activity, however it is recommended that the product be aliquoted into single use volumes so that freeze-thaw cycles will not be required. GCT-CM is stable after 3 days at 4°C, room temperature (22°C), or 37°C.

**SUGGESTIONS FOR USE**

Growth of Hematopoietic Cells

IS Giant Cell Tumor-Conditioned Medium 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 three factors contained within IS GCT-CM) are required to obtain the maximum number of granulocyte and macrophage colonies in 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 is evidenced by increased colony size and more colonies than the expected sum of the number of colonies obtained with each of the factors alone (6).

Suggested concentration for use:
IS GCT-CM should be titrated to find the optimal 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 (CFU) obtained from human bone marrow progenitor cells is typically determined from a culture of 1 - 10^6 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 form 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 which were obtained when the patient was in remission after chemotherapy.

Suggested concentration for use:
IS GCT-CM should be titrated to find the optimal concentration for each application and set of conditions. Typical concentrations of GCT-CM for maximum stimulation of the 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 improves the length and morphology of chromosomes and increases the mitotic index of specimens obtained with normal karyotypes, or abnormal karyotypes of diagnosed lymphoid leukemia, preleukemia, or chronic myelogenous leukemia patients (10).

Suggested concentration for use:
IS GCT-CM should be titrated to find the optimal concentration for each application and set of conditions. A 10% (v/v) concentration of GCT-CM used during the 24 hour culturing of the bone marrow specimens before slide preparation enhanced 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 coculture 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 improve 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 optimal 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) 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 titrated to find the optimal 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**

Reagents:
IS Giant Cell Tumor-Conditioned Medium
Mice (BALB/c or other strain), 3-7 months old
clMM: 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 x 10^4 and 1 x 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 100 µL 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.**

**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 through a capsule filter with a pore size of 0.2 um.

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

Mycoplasma detection in growth agar and broth.

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

Biological activity testing Chang-BMC test

**QUALITY CONTROL**

Quality Control of GCT-CM includes: pH Osmolality Bioburden

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