IS GIANT CELL TUMOR CONDITIONED MEDIUM (GCT-CM)

Catalog Nos. 91006-50 mL

INTENDED USE
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.

STABILITY AND STORAGE
IS GCT-CM is stable when stored unopened at -10°C for up to two (2) years from the date of manufacture. Store bottle 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 (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 (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 (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 (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 (all present in IS GCT-CM) is required to obtain the maximum number of granulocyte and macrophage colonies. 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 hybridoma cell lines.

Suggested concentration for use:
IS GCT-CM should be titrated to find the optimum concentration for each application and set of conditions. A concentration of 5 to 10% (v/v) 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, 5x10⁶ 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) IS GCT-CM and between 4 x 10⁶ and 1 x 10⁷ 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% CO₂/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.

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 recombiant 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. A concentration of 10% (v/v) IS GCT-CM used during the 24 hour culturing of bone marrow specimens before slide preparation was shown to enhance the cytogenetic analyses (10).

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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 hybridoma cell lines.

Suggested concentration for use:
IS GCT-CM should be titrated 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.
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 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, Tryptase 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.

References


