



# GIST-T1 Culture Kit

(Human Cell Line)

## !Caution!

- See attached “Please Read BEFORE USE”
- Please use the composed media (Cat.no# PMC-GISTM-COS) for the culture of GIST-T1. Warranty will not be covered when using other medium.
- We recommend you always wear gloves and safety glasses when handling GIST-T1.
- We DO NOT guarantee the GIST-T1 quality when cryopreserved at customer's laboratory.
- Based on the license policies of Techno network Shikoku and Kochi University, GIST-T1 is prohibited to provide (distribution, lending, transfer, licensing, etc.) to any third parties.

## Principle

Gastrointestinal stromal tumors (GISTs) are one of the submucosal tumor, occur in the stomach, the small intestine and the esophagus, unlike most gastrointestinal tumors. GISTs are considered to arise from the interstitial cells of Cajal, the pacemaker cells of the gut.

The GIST-T1 is a cell line derived from GISTs of the stomach in a Japanese woman and established by Takahiro Taguchi; associate professor, Graduate School of Integrated Arts and Sciences, Kochi-University, Kochi, Japan.

## Components

Product Name	Quantity	Amount	Storage Conditions	Stability
GIST-T1, cryopreserved	1.0x10 <sup>6</sup> cells/vial	1	Liquid Nitrogen vapor phase	6 months
Culture Medium	250 mL	1	-20°C Freezer	6 months
			4°C	3 months

**\*Shipping: dry ice**

**Culture Medium components: DMEM, FBS, antibiotic, etc.**

## Materials required but not provided

- Variable volume pipettes
- Culture vessels
- 0.25% Trypsin
- HBSS or PBS(-)

## General Information

<b>Organism</b>	<i>Homo sapiens</i> , human
<b>Tissue</b>	Stomach
<b>Cultural Properties</b>	Adherent
<b>Biosafety Level</b>	1
<b>Gender</b>	Female
<b>Ethnicity</b>	Asian
<b>Virus Check</b>	HIV-1(-), HTLV-1(-), HBV(-), HCV(-), T.pallidum(-)
<b>Quality Check</b>	Mycoplasma (-)

## Protocol

### A) Unpacking & Storage conditions

- 1) Check all containers for leakage or breakage.
- 2) Remove the frozen cells from the dry ice packaging and immediately transfer the cells into liquid nitrogen vapor phase, until ready to use.

NOTE: It should be stored in liquid nitrogen vapor phase and not at -70°C or higher. Storage at -70°C or higher will result in loss of viability.

### B) Thawing of Cells

- 1) Prepare a 100mm dish. NOTE: 100mm dish is recommended.
- 2) Prepare a conical tube (for 15mL) added 10mL of culture medium.
- 3) Pre-heat water bath to 37°C.
- 4) Carefully remove the vial(s) from liquid nitrogen.  
NOTE: When your water bath is apart from Liquid N2 tank, place vial(s) in dry ice during the transport.
- 5) Thaw the vial by gentle agitation in a **37°C** water bath for **100sec. ± 10sec.**  
NOTE: Thaw cells gently and quickly (do not place vial(s) longer than 2minutes).  
Immerse the vial(s) into a water bath (37°C) just up to the screw cap.  
NOTE: Remove vial(s) from water bath when an ice pellet becomes c.a. 3~5mm size.
- 6) Transfer the vial(s) into a laminar flow hood. Before opening, wipe the outside of the vial with 70% ethanol.
- 7) Gently transfer the thawed cell suspension (1mL) into 10 mL of culture medium.
- 8) Rinse vial again with 1mL medium and back to 15mL tube.
- 9) Centrifuge the cell suspension at approximately 200 xg for 5 minutes at 4°C
- 10) Aspirate the supernatant without disrupting the pellet and re-suspend the cells in 10mL of culture medium.
- 11) Transfer the cell suspension to 100mm dish and incubate the cells in 37°C, 5% CO<sub>2</sub> incubator.
- 12) Replace the medium with fresh pre-warmed culture medium every 2 to 3 days.

### C) Subculturing

NOTE: Allow culture medium, HBSS (or PBS (-)), and 0.25% Trypsin to room temperature before use.

- 1) When the cells reach 70 -90% of confluent, they should be subcultured.
- 2) Aspirate the medium. Rinse the dish with 10mL of HBSS or PBS (-).
- 3) Add 1mL of 0.25% Trypsin, then incubate at 37°C for 4-6 minutes.
- 4) Add 10mL of culture medium and disperse the cells with gentle pipetting.
- 5) Transfer the cell suspension to conical tube and centrifuge at 200 ×g for 5 minutes at 4°C.
- 6) Aspirate the supernatant without disrupting the pellet and resuspend the cells in 10mL of culture medium.
- 7) Dilute the cell suspension by adding culture medium to 1:6 to 1:8.
- 8) Transfer the cell suspension to new 100mm dish and incubate the cells in 37°C, 5% CO<sub>2</sub> incubator.
- 9) Replace the medium with fresh pre-warmed culture medium every 2 to 3 days.
- 10) Culture the cells until the required density (70 -90% of confluent; Fig 1, C) is reached.

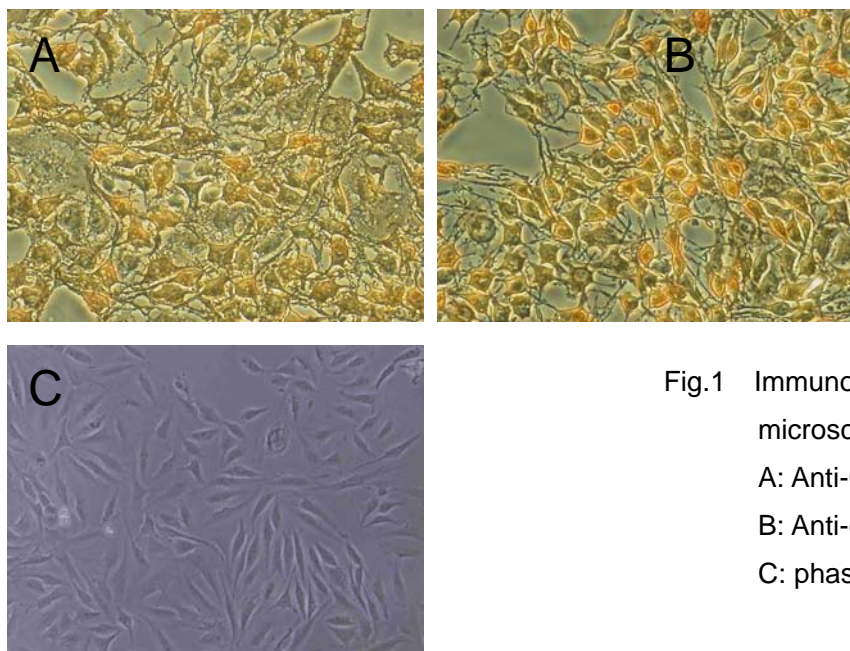


Fig.1 Immunohistochemical and phase-contrast microscopic observation

A: Anti-CD34

B: Anti-c-kit

C: phase-contrast microscopic observation

### References

- 1) Takahiro Taguchi, Hiroshi Sonobe, and Kazunari Yuri. et al. Conventional and Molecular Cytogenetic Characterization of a New Human Cell Line, GIST-T1, Established from Gastrointestinal Stromal Tumor. Lab Invest. 2002 May;82(5):663-5.

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