Cat. # WK551S

For Research Use

TaKaRa

GT-T551 Culture medium, 1L Bottle

Product Manual

v201602

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I. Description

GT-T551 Culture medium is used for extended culture of human T lymphocytes as well as during T cell transduction by retroviral or lentiviral vectors. The medium contains only human serum albumin (pharmaceutical grade) and recombinant human insulin; no additional proteins or growth factors are added. GT-T551 Culture medium is tested to ensure it is sterile, free of endotoxins and mycoplasma, and promotes adequate lymphocyte proliferation activity. Correct pH and osmolality are also verified.

For T cell expansion, peripheral blood mononuclear cells (PBMCs) are typically used as starting material. Further T cell purification from PBMCs is not needed for T cell expansion. PBMCs can be separated by density gradient centrifugation from heparinized blood or by apheresis. Alternatively, it can be purchased from vendors.

II. Component

GT-T551 Culture medium, 1L Bottle	1,000 mL

III. Storage

2℃ to 8℃

IV. Note

GT-T551 Culture medium is supplemented with human serum albumin, human insulin, L-glutamine, and streptomycin, and it can be directly used for T cell culture. Although GT-T551 Culture medium is capable of supporting T cell proliferation without adding serum and plasma, cell growth may be enhanced by addition of these components in the medium. T cells can be cultured in GT-T551 Culture medium with cytokines (IL-2, IL-15, etc.) in an anti-CD3 antibody-stimulated state.[1-3] During anti-CD3 antibody stimulation, the presence of RetroNectin® enhances the proliferation of T cells.[4-8] The expanded cell population shows a higher proportion of naïve T cells than anti-CD3 antibody stimulation alone (see page 6).

V. Application

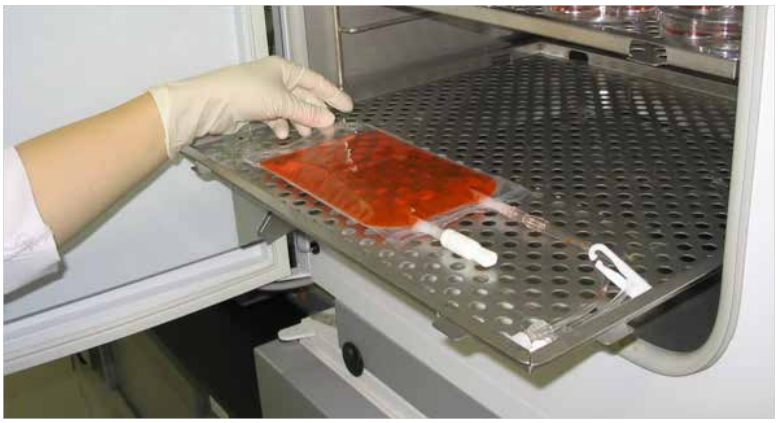
GT-T551 Culture medium was used during T cell expansion with RetroNectin (Cat. #T100A/B) stimulation in a gas-permeable culture bag.

Note: The method of T cell expansion by RetroNectin stimulation requires a license from Takara Bio Inc. for uses other than research.

【 Method 】

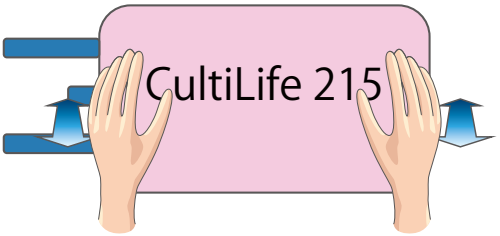
1) Coat CultiLife™ 215 Culture bag with anti-CD3 monoclonal antibody and RetroNectin (Set-up; Day 0)
   - Prepare 30 mL of PBS containing anti-CD3 mAb (5 μg/mL) and RetroNectin (25 μg/mL).
   - Pour the solution into a CultiLife 215 Culture bag.
   - Incubate for 2 - 5 hours at 37℃ in a 5% CO_2 incubator.
   - Remove the solution and gently rinse the inside of the bag with 30 mL of PBS.
   - Repeat the rinse step twice.
     Note: On the last rinse, remove the wash solution just before adding PBMCs.

2) Add PBMCs
   - Suspend about 3 x 10^7 PBMCs in 30 - 50 mL of GT-T551 Culture medium containing human serum or plasma in a final concentration of 0.5 - 1.0%.
   - Pour the PBMC suspension into the anti-CD3 mAb-coated and RetroNectin-coated CultiLife 215 Culture bag prepared during Step 1).
   - Add GT-T551 Culture medium up to 200 - 300 mL and IL-2 (final concentration of 200 - 1,000 U/mL).
   - Culture at 37℃ in a 5% CO_2 incubator.



3) Passage at Day 4
- Collect all of the cell suspension from the CultiLife 215 Culture bag using the method described below and transfer to a new GT-T610 (CultiLife Eva) Culture bag (Cat. #FU0010).*

  Note: Cells cultured in the RetroNectin-coated bag adhere to the bag. Cells can be suspended by rocking the culture bag back and forth with both hands about 50 times as shown below.



- Confirm by microscopy that no cells remain in the CultiLife 215 Culture bag.
- Add an appropriate volume of GT-T551 Culture medium and IL-2 (final concentration of 200 - 1,000 U/mL).

  Note: Max volume of GT-T610 (CultiLife Eva) Culture bag is 1,000 mL.
- Culture at 37℃ in a 5% CO_2 incubator.

  * At this time, scale-up is possible by passaging the cells into more culture bags according to the rate of cell growth and the desired endpoint cell number.

4) Harvest cells
- Suspend cells by rocking the Culture bag about 20 - 30 times using the same method described in Step 3) for the CultiLife 215 Culture bag.
- Collect the cell suspension from the GT-T610 (CultiLife Eva) Culture bag.
- Wash cells by centrifugation, or if the culture volume is large, by using a cell washing apparatus such as the Cell Server.

  * PBS or saline containing 0.1% human serum albumin can be used for the wash solution.

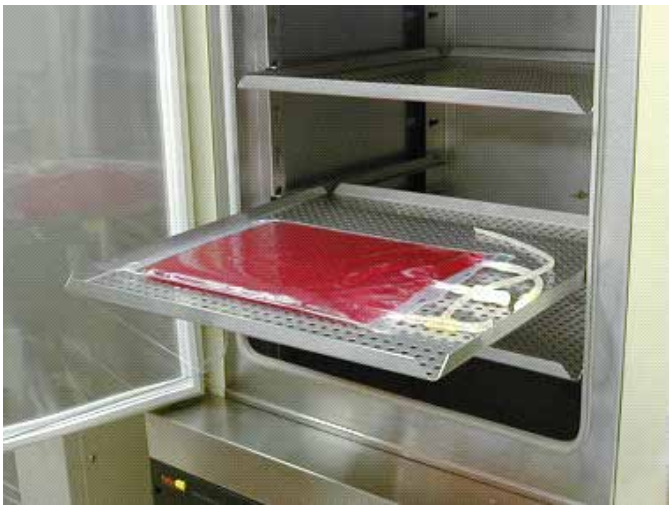


Table 1. Example of culture

	Day 0	Day 4	Day 7	Day 10
PBMC number	3 x 10^7 cells			
Culture volume	300 mL	500 mL x 5 *3	1,000 mL x 5 *4	
Culture area	215 cm^2 *1	640 cm^2 *2 x 5		
	CultiLife 215 x 1	GT-T610 x 5		Harvest

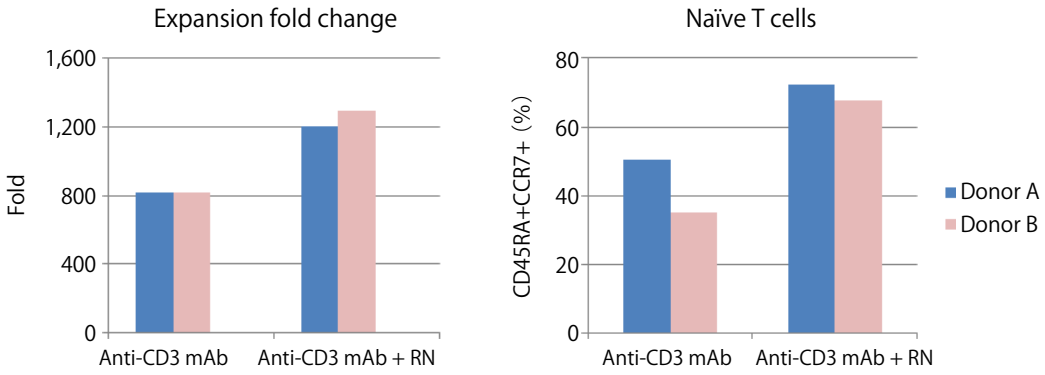
*1: Culture area of one CultiLife 215 Culture bag is 215 cm^2.
*2: Culture area of one GT-T610 (CultiLife Eva) Culture bag is 640 cm^2.
*3: If the concentration of the cell suspension is less than 4 x 10^5 cells/mL at Day 4, it is recommended to passage on Day 5.
If passaging on Day 4, use a cell suspension concentration of at least 5 x 10^4 cells/mL.
*4: At this stage, passage using a cell suspension concentration of at least 5 x 10^5 cells/mL.

【 Example of T cell expansion using healthy donor PBMCs 】

Method

PBMCs and autologous plasma were prepared using 50 mL of blood from two healthy volunteers on the basis of informed consent. The effect of RetroNectin (RN) co-stimulation on T cell expansion was tested using the culture system above. Heat-inactivated plasma at a final concentration of 0.6% was added to the GT-T551 Culture medium at Day 0 and Day 4.

Results



RetroNectin co-stimulation resulted in a dramatic increase in T cell expansion and the population contained a high proportion of naïve T cells.

VI. References

1) Wang, Y., *et al*. (2013) CIK cells from recurrent or refractory AML patients can be efficiently expanded in vitro and used for reduction of leukemic blasts in vivo. *Exp Hematol*. **41**(3): 241-252.

2) Ai, Y. Q., *et al*. (2014) The clinical effects of dendritic cell vaccines combined with cytokine-induced killer cells intraperitoneal injected on patients with malignant ascites. *Int J Clin Exp Med*. **7**(11): 4272-4281.

3) Dodo, K. *et al*. (2014) An efficient large-scale retroviral transduction method involving preloading the vector into a RetroNectin-coated bag with low-temperature shaking. *PLoS ONE*. **9**(1): e86275.

4) Yu, S. S, *et al*. (2008) In vivo persistence of genetically modified T cells generated ex vivo using the fibronectin CH296 stimulation method. *Cancer Gene Ther*. **15**(8): 508-516.

5) Ishikawa, T., *et al*. (2014) Phase I clinical trial of fibronectin CH296-stimulated T cell therapy in patients with advanced cancer. *PLoS ONE*. **9**(1): e83786.

6) Hosoi, H., *et al*. (2014) Stimulation through very late antigen-4 and -5 improves the multifunctionality and memory formation of $CD8^+$ T cells. *Eur J Immunol*. **44**(6): 1747-1758.

7) Sakamoto, N., *et al*. (2015) Phase I clinical trial of autologous NK cell therapy using novel expansion method in patients with advanced digestive cancer. *J Transl Med*. **13**: 277.

8) Li, W., *et al*. (2015) Efficacy of RetroNectin-activated cytokine-induced killer cell therapy in metastatic brain tumor patients. *Oncol Res Treat*. **38**(4): 160-165.

VII. Related Products

RetroNectin® Recombinant Human Fibronectin Fragment (Cat. #T100A/B)
Anti-CD3 mAb GMP grade Anti-CD3 monoclonal antibody (Clone OKT3) (Cat. #T210)
CultiLife™ 215 Culture bag (Cat.#FU0005)
GT-T610 (CultiLife™ Eva) Culture bag (Cat. #FU0010)

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