Cat. # WK552S

For Research Use

TaKaRa

LymphoONE™ T-Cell Expansion Xeno-Free Medium, 1L Bottle

Product Manual

v201903

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I. Description

LymphoONE T-Cell Expansion Xeno-Free Medium is a suitable medium for human T-lymphocyte (T-cell) expansion culture and gene transduction into T lymphocytes using retroviral and lentiviral vectors. The medium does not contain proteins or growth factors other than human serum albumin (pharmaceutical-grade) and recombinant human insulin. As compared to our previous product, GT-T551 Culture medium (Cat. # WK551S), this product improves T-cell proliferation rates without serum or plasma through optimization of medium components.
Various tests are performed for all lots of LymphoONE T-Cell Expansion Xeno-Free Medium such as sterility, mycoplasma, endotoxin, and lymphocyte proliferation tests, etc. In addition, pH and osmotic pressure have been confirmed to be within the expected ranges.
As T-cell expansion culture has begun to use peripheral blood mononuclear cells (PBMCs) as a starting material, isolation of T cells is not specifically required. PBMCs are available as commercial cells and can be obtained from peripheral blood through density gradient centrifugation, etc.

II. Components

LymphoONE T-Cell Expansion Xeno-Free Medium, 1L Bottle	1,000 ml

III. Storage

2 - 8℃

IV. Method of Use

LymphoONE T-Cell Expansion Xeno-Free Medium contains human serum albumin, human insulin, L-glutamine, and streptomycin; therefore you can directly use it for T-cell culture. LymphoONE T-Cell Expansion Xeno-Free Medium can be used for T-cell expansion culture without adding serum or plasma. However, there are cases where the cell proliferation rate increases by adding serum and plasma.
T cells can be propagated by culturing in LymphoONE T-Cell Expansion Xeno-Free Medium with cytokines (IL-2 or IL-15 etc.), after stimulating with anti-CD3 mAb[1-3]. T-cell proliferation can be enhanced when T cells are stimulated by RetroNectin® reagent with anti-CD3 mAb. In addition, a proliferated cell population contains more naïve T cells, as compared to a single stimulation of anti-CD3 mAb[4-8].

V. Application: T-Cell Expansion Culture by RetroNectin Costimulation

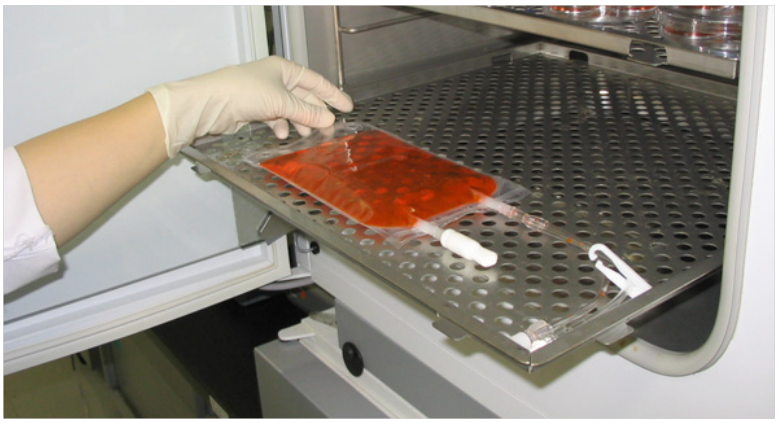
T cells were cultured to expand cell number via RetroNectin costimulation using LymphoONE T-Cell Expansion Xeno-Free Medium and a gas-permeable culture bag.

Note: You are required to have a license agreement contract with Takara Bio Inc., when you perform T-cell expansion culture using RetroNectin reagent for a purpose other than research.

[Method]

1) Coating a CultiLife™ 215 Culture bag (Cat. # FU0005) with Anti-CD3 mAb (Cat. # T210) and RetroNectin reagent (Cat. # T100A/B, T202) (Setup, Day 0)
    1. Prepare 30 ml of PBS containing Anti-CD3 mAb (5 μg/ml) and RetroNectin reagent (25 μg/ml).
    2. Add the entire volume to a CultiLife 215 Culture bag.
    3. Coat the culture bag by incubating at 37℃, 5% CO_2 for 2 to 5 hours.
    4. Remove the entire solution from the bag.
    5. Wash the bag with 30 ml of PBS, 3 times.
       Note: Remove the last of the PBS just before seeding PBMCs.

2) Seeding of PBMCs (Day 0)
    1. Suspend about 3 x 10^7 PBMCs in 30 to 50 ml of LymphoONE T-Cell Expansion Xeno-Free Medium containing 0 to 1% human serum or plasma.
    2. Add the total volume of PBMC suspension to the coated CultiLife 215 Culture bag (see Method-1, above).
    3. Add LymphoONE T-Cell Expansion Xeno-Free Medium with 0 to 1% human serum or plasma to the bag until the liquid volume reaches 200–300 ml. Then add IL-2 to a final concentration of 200 to 1000 U/ml.
    4. Culture in an incubator at 37℃, 5% CO_2 for four days.

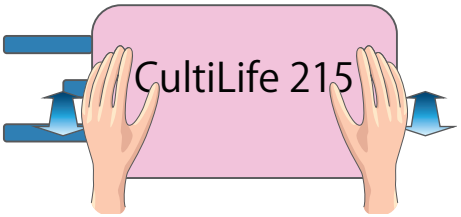


3) Subculture (Day 4)

1. Collect cells from the CultiLife 215 Culture bag using the following method, and transfer them to a new GT-T610 (CultiLife Eva) Culture bag (Cat. # FU0010).

   [Cell suspension method]

   Cells cultured in a bag coated with RetroNectin will adhere strongly to inside of the bag. Hold the bag with both hands and rock it back and forth about 50 times to suspend the cells in the culture medium, as shown in the figure below.



2. Use a microscope to check that there are almost no cells left in the CultiLife 215 Culture bag.
   Note: If some cells still remain in the bag, you can add an appropriate amount of medium to the bag and collect them in the same way as above.

3. Add an appropriate amount of LymphoONE T-Cell Expansion Xeno-Free Medium and IL-2 (to a final concentration of 200–1000 U per 1 ml of culture medium).
   Note: At this point, you can increase the number of GT-T610 Culture bags as necessary (see Table 1). The maximum capacity of a GT-T610 Culture bag is 1,000 ml.

4. Continue cell culture in an incubator at 37°C, 5% CO_2.
   Note: Incubate for 10 to 14 days while properly diluting cells as cell number increases (refer to Table 1).

4) Collection of cells (Day 10 to 14)

1. Suspend cells by rocking the medium in the GT-T610 Culture bag 20 to 30 times in the same way as in method-3, above.
2. Collect the cell suspension from the bag.
3. Wash the cells by centrifugation or using an appropriate cell cleaning apparatus.
   Note: PBS containing 0.1% human serum albumin or physiological saline solution is appropriate for use as a cleaning solution.

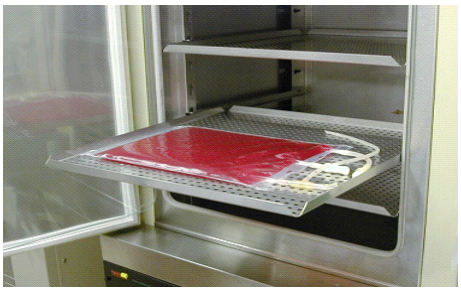


Table 1. Example culture specifications

	Day 0	Day 4	Day 7	Day 10
Cell number	3 x 10^7 cells			
Medium 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		Cell collection

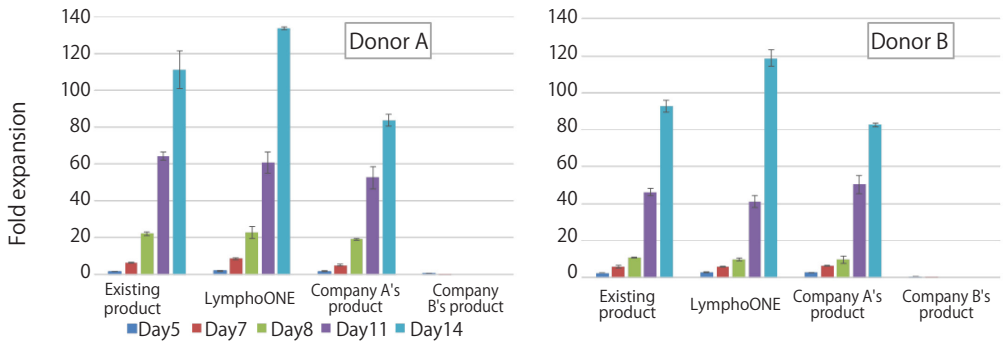
*1 The culture area of a CultiLife 215 Culture bag is 215 cm^2.
*2 The culture area of a GT-T610 (CultiLife Eva) Culture bag is 640 cm^2.
*3 When cell concentration is less than 4 x 10^5 cells/ml on Day 4, continue to culture for one more day. On Day 4, subculture to a cell concentration of more than 5 x 10^4 cells/ml.
*4 At this point, subculture so that cell concentration reaches more than 5 x 10^5 cells/ml.

[Comparison of media in T-lymphocyte expansion culture]

Method

Peripheral blood mononuclear cells (PBMCs) and blood plasma were isolated from blood collected from two healthy donors, from whom informed consent was obtained.
PBMCs were cultured for 4 days under a stimulation condition in a CultiLife215 Culture bag with Anti-CD3 mAb, and then further cultured for 10 days while appropriately diluting the cells with lymphocyte culture media with IL-2.

Results



LymphoONE T-Cell Expansion Xeno-Free Medium showed a high expansion rate even without serum (human AB serum), and it was confirmed that LymphoONE T-Cell Expansion Xeno-Free Medium has a better proliferation performance than the previous product and competitors' products.

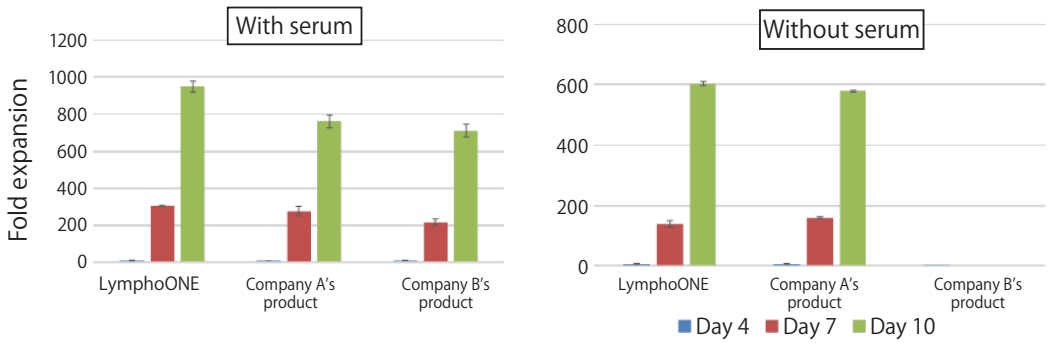
[T-cell expansion culture by RetroNectin costimulation]

Method

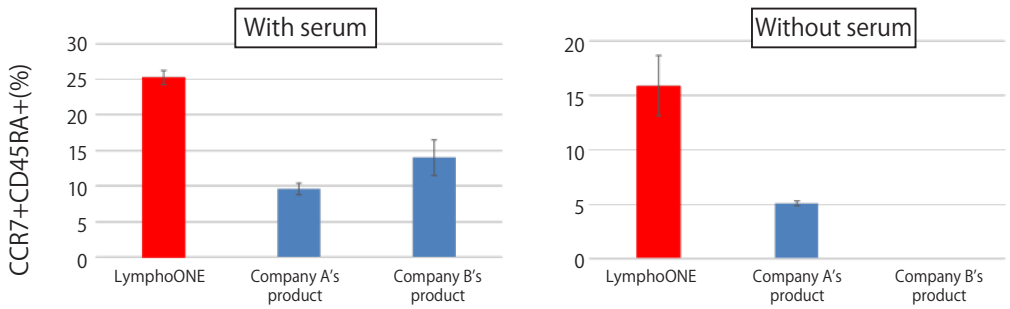
Human peripheral blood mononuclear cells (PBMCs) were cultured under a costimulation condition with RetroNectin in a CultiLife215 Culture bag with Anti-CD3 mAb for 4 days. Then they were cultured using various lymphocyte culture media with IL-2 for 10 days, with and without serum (human AB serum). Serum was added at 1% from Day 0 to 7, then at 0.5% from Day 7 to 10.

Results

Expansion culture rate



Naïve T cell ratio



A high expansion culture rate was observed from LymphoONE T-Cell Expansion Xeno-Free Medium even without serum (human AB serum). It was confirmed that the cell population cultured in this medium had a high number of naïve T cells.

VI. Reference

1) Wang, Y., *et al*. 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*. (2013) **41**(3): 241-252.

2) Ai, Y. Q., *et al*. 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*. (2014) **7**(11): 4272-4281.

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

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

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

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

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

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

VI. Related Products

RetroNectin® (Recombinant Human Fibronectin Fragment) (Cat. # T100A/B)
RetroNectin® GMP grade Recombinant Human Fibronectin Fragment CH-296 (Cat. # T202)
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)

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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