ALyS505NK-AC and ALyS505NK-EX

Instruction for use

CELL SCIENCE & TECHNOLOGY INSTITUTE INC.

ver. 8

Product Description

ALyS505NK-AC and ALyS505NK-EX is a medium for culture of human NK cells. ALyS505NK-AC and ALyS505NK-EX is a Xeno-free* medium.

* Xeno-free : It contains human derived component. Any other animal derived component free.

Product	Catalog Number (NIPRO/CSTI)	Components	Volume	Container	Storage
ALyS505NK-AC	87-554/01600P02	NK cell culture medium for activation without IL-2	200 mL	PET bottle	2-8 ℃ ; Protect from Light
ALyS505NK-AC1000	87-555/01610P02	NK cell culture medium for activation with IL-2 1000 IU/mL	200 mL	PET bottle	2-8 ℃ ; Protect from Light
ALyS505NK-EX	87-556/01400P10	NK cell culture medium for expansion without IL-2	1000 mL	PET bottle	2-8 ℃ ; Protect from Light
	87-558/01400C10		1000 mL	Culture Bag	2-8 ℃ ; Protect from Light
ALyS505NK-EX1000	87-557/01410P10	NK cell culture medium for expansion with IL-2 1000 IU/mL	1000 mL	PET bottle	2-8 ℃ ; Protect from Light
	87-559/01410C10		1000 mL	Culture Bag	2-8 ℃ ; Protect from Light
Related Product	**Catalog Number (NIPRO/CSTI)**	**Components**	**Volume**	**Container**	**Storage**
PBS(-)	87-949/1102P05	Dulbecco's phosphate buffered saline	500 mL	PET bottle	2-8 ℃
	87-972/1102P10		1000 mL	PET bottle	2-8 ℃
Lymactin-NK	87-025/6002T01	Anti-Her2 monoclonal antybody	1 mL	tube	below -20 ℃

Storage

ALyS505NK-AC and ALyS505NK-EX instructions: upon arrival, store ALyS505NK-AC and ALyS505NK-EX protected from light at 2℃ to 8℃.

Preparation of Culture Media

1. Decontaminate the external surfaces of the vessel with 70% v/v ethanol.

2. Please add IL-2 (1000 IU/mL) into ALyS505NK-AC (Cat.No.01600P02) and ALyS505NK-EX (Cat.No.01400P10) before use.

* Recommend to make necessary volume of the medium just before use.

Preparation of Antibody coated Flask

1. Add 4mL of PBS(-) and 1mL of **Lymactin-NK** or Anti-Her2 MAb stock solution into 75 cm^2 Culture Flask.

2. Gently shake the flask and spread the solution on the surface of Culture Flask.

3. Incubate for more than 2 hr at room temperature and store at 4℃ until use.
4. Remove the MAb solution.
5. Wash the flask twice with PBS(-). The washed flask should be used immediately.

Separation of mononuclear cells from blood

1. Collect peripheral blood into a tube containing anticoagulant (ex. Heparin)
2. Carefully layer 20-30 mL of the blood over 15 mL Lymphoprep. Avoid mixing of blood and Lymphoprep.
3. Centrifuge at 800 x g for 20 minutes at room temperature (approximately 20 ℃) using a swing-rotor. If the blood is stored for more than 2 hours, extend the centrifugation time to 30 minutes.
4. After centrifugation, the blood is separated into 4 blocks of Plasma (upper layer), Mononuclear cells between Plasma and Separation fluid (2nd layer), Lymphoprep (3rd layer) and red blood cell (bottom Layer).

Preparation of Heat Inactivated Human Plasma

1. Collect the plasma layer into a sterilized centrifuge vessel by pipette.
   "Should be careful not to take the second Mononuclear cells layer."
2. Heat the plasma at 56 ˚C for 30 min.
3. Centrifuge at 1200 x g for 10 min. at room temperature.
4. Collect supernatant into a sterilized vessel by pipette and store in refrigerator until use.

Preparation of Peripheral blood Mononuclear cells (PBMC)

1. Collect the Mononuclear Cells of 2nd layer using a pipette into a sterilized centrifuge vessel.
2. Dilute the collected fraction with PBS(-) and pellet the cells by centrifugation for 10 min. at 500 x g.
3. Remove supernatant by aspiration.
4. Wash the cells with PBS(-) and pellet the cells by centrifugation for 10 minutes at 500 x g.
5. Remove supernatant by aspiration.
6. Repeat 4. and 5..

Methods of NK-Cell culture

1. Re-suspend PBMC with 20 to 60mL of ALyS505NK-AC1000 (1000 IU/mL IL-2)(ACTM) **containing 5 to 10% heat inactivation plasma** at the cell density of more than $1x10^6$ cells/mL.(Do not seed the cells below the density "$1x10^6$ cells/mL")
2. Seed the cell suspension into required number of the antibody coated flask (20 mL for each T-75cm^2 flask).
3. Incubate the cells at 37℃ in 5 % CO_2/air incubator and culture them according to a culture schedule described below.
4. Add ACTM into culture flasks at day 3rd, 5th
5. The cell suspension transfer into a Culture Bag with ALyS505NK-EX containing 1,000 IU/mL IL-2 (EXPM) at day 6th to 8th.
6. Expand the culture bags depending on the culture condition.
7. Harvest the cells at day14th

Methods of Cell harvest

1. After 14 days culture, collect the all cell suspension into sterilized centrifuge bottle, and the cells precipitate by centrifugation at 500 x g for 10 minutes.
2. Wash the cells twice with Ringer solution by

repeat centrifugation.

3. Re-suspend the cells with Ringer solution or Saline containing 0.1% Human serum Albumin.

Schedule of NK Cell culture

Day	Vessel	Number of Vessel	Media	Add heat inactivated human plasma	Add New medium	Total Vol.	Remarks
				(mL)	(mL)	(mL)	
-1	Flask T-75	1	-	-	-	-	
0	Flask T-75	1	ACTM	2	20	20/Flask	*1
3	Flask T-75	1	ACTM	-	40	60/Flask	
5	FlaskT-225	1	ACTM	10	140	200/Flask	*2
7	Culture Bag	1	EXPM	-	400	600/Flask	*3
9	Culture Bag	1	EXPM	-	700	1,300/Bag	
11	Culture Bag	2	EXPM	-	700	1,000/Bag	*4
14	Culture Bag	2		-	-	1,000/Bag	*5

ACTM : ALyS505NK-AC1000 (IL-2 1000 IU/mL), EXPM : ALyS505NK-EX1000 (IL-2 1000 IU/mL)

*1 Cell Density at seeding($1x10^6$ cells/mL)
*2 Caution : Do not add the plasma over 10 mL
*3 Transfer the cell suspension into a Culture Bag
*4 Expand a bag to two bags
*5 Cell Harvest

Flow chart of NK Cell culture

Pre-coat the Culture Flask with Antibody (**Lymactin-NK** or Anti-Her2 MAb).

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Isolate the Mononuclear cells (PBMC) by density gradient.

⇩

Seed the cells into the antibody coated flask, with Activation medium (ACTM).

⇩ 2 days

Add new medium and continue the culture based on culture schedule

⇩ 4~ 6 days

Transfer the cells into a Culture Bag Medium (EXPM)

⇩ 3 ~ 4 days

Expand to Double Culture Bag (EXPM)

⇩ 3 ~ 4 days

Cell Harvest

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