

# ALyS505NK-AC and ALyS505NK-EX

## Instruction for use

### 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 : Contains human derived component, and free of other animal derived component.

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

### Storage

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

### Preparation of Culture Media

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

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

\* Recommend making 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 75cm<sup>2</sup>  
Culture Flask.

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

3. Incubate for more than 2 hours at room temperature and store at 4°C 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°C) 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 layers. Plasma (upper layer), Mononuclear cells (2<sup>nd</sup> layer) Separation fluid (3<sup>rd</sup> layer), Lymphoprep (4<sup>th</sup> 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.  
\*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 2<sup>nd</sup> 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  $1 \times 10^6$  cells/mL. (Do not seed the cells below the density " $1 \times 10^6$  cells/mL")
2. Seed the cell suspension into required number of the antibody coated flask (20 mL for each T-75cm<sup>2</sup> flask).
3. Incubate the cells at 37°C in 5 % CO<sub>2</sub>/air incubator and culture them according to a culture schedule described below.
4. Add ACTM into culture flasks at day 3 and 5.
5. Transfer the cell suspension into a culture Bag with ALyS505NK-EX containing 1,000 IU/mL IL-2 (EXPM) at day 6 to 8.
6. Use additional culture bags depending on the culture condition.
7. Harvest the cells at day 14.

### Methods of Cell harvest

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

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   | Flask T-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 ( $1 \times 10^6$  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**

