ALyS505N

Instruction for use

CELL SCIENCE & TECHNOLOGY INSTITUTE INC.

ver. 11

Product Description

ALyS505N is a medium for culture of lymphokine activated killer cell (LAK). ALyS505N is a Xeno-free* medium.

* Xeno-free : Contains human derived component, and free of other animal derived component.

Product	Catalog Number (NIPRO/CSTI)	Components	Volume	Container	Storage
ALyS505N-0	87-661/1020P10	Serum-free medium for Lymphocyte without IL-2	1000 mL	PET bottle	2-8 ℃ ; Protect from Light
	87-669/1020C10			Culture Bag	
ALyS505N-175	87-654/10217P10	Serum-free medium for Lymphocyte with IL-2 175IU/mL	1000 mL	PET bottle	2-8 ℃ ; Protect from Light
	87-598/10217C10			Culture Bag	
ALyS505N-7	87-666/1027P10	Serum-free medium for Lymphocyte with IL-2 700IU/mL	1000 mL	PET bottle	2-8 ℃ ; Protect from Light
ALyS505N-10	87-676/10210P10	Serum-free medium for Lymphocyte with IL-2 1000IU/mL	1000 mL	PET bottle	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	1-30 ℃
	87-972/1102P10		1000 mL	PET bottle	1-30 ℃
Lymactin-T	87-984/6001T01	Anti-CD3 monoclonal antibody	1 mL	tube	below -20 ℃

Storage

ALyS505N instructions: upon arrival, store ALyS505N 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 into ALyS505N-0 (Cat.No.1020P10, 1020C10) before use.

* Recommend making necessary volume of the medium just before use.

Preparation of Antibody coated Flask

1. Add 10 mL of PBS(-) and 0.050 mL of **Lymactin-T** or Anti-CD3 MAb stock solution into 225 cm^2 Suspension Culture Flask.
2. Gently shake the flask and spread the solution on the surface.
3. Incubate for more than 1 hour 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 layers. Plasma (upper layer), Mononuclear cells (2^{nd} layer), Separation fluid (3^{rd} layer), Lymphoprep (4^{th} 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^{nd} 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 LAK-Cell culture

1. Re-suspend PBMC with about 50 mL of ALyS505N-175 or ALyS505N-7(**containing 8 to 10% heat inactivation plasma** at the cell density of about $2x10^5$ cells/mL)
2. Seed the cell suspension into the antibody coated 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 Medium into culture flasks at day 3 and 5.
5. Transfer the cell suspension into a culture Bag with ALyS505N-175 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 LAK Cell culture

Day	Vessel	Number of Vessel	Add heat inactivated human plasma (mL)	Add New medium (mL)	Total Vol. (mL)	Remarks
-1	Flask T-225 *5	1	-	-	-	
0	Flask T-225	1	5	50	50	*1
3	Flask T-225	1	-	50	100	
5	Flask T-225	1	-	100	200	
7	Culture Bag	1	-	1000	1200	*2
9	Culture Bag	2	-	1000	1100/Bag	*3
11	Culture Bag	4	-	2000	1,050/Bag	*3
14	Culture Bag	4	-	-	1,050/Bag	*4

*1 Cell Density at seeding($2x10^5$ cells/mL)
*2 Transfer the cell suspension into a Culture Bag
*3 Expand a bag to two bags
*4 Cell Harvest
*5 Suspension culture flask

Flow chart of LAK Cell culture

Pre-coat the Culture Flask with Antibody (**Lymactin-T** or Anti-CD3 MAb).

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Isolate the Mononuclear cells (PBMC) by density gradient.

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Seed the cells into the antibody coated flask, with ALyS505N(contained IL-2).

⇩ 2 days

Add new medium and continue the culture based on culture schedule

⇩ 4~ 6 days

Transfer the cells into a Culture Bag Medium

⇩ 2 ~ 3 days

Expand a culture bag to two bags

⇩ 2 ~ 3 days

Expand two culture bags to four bags

⇩ 2 ~ 3 days

Cell Harvest

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CD-25178