Instructions for Using CIK Culture Medium KIT

I. Product Information:

Product Name	Product Description	Product NO.	Amount	Price (USD)
Culture Medium for CIK	CIK Serum-free Culture Medium (Xeno-free, with phenol red)	N107-500	500ml	Inquiry
		N107-1000	1000ml	
Culture Medium KIT for CIK	CIK Cell Serum-free Culture Medium （Xeno-free）	N107-1000	1000ml*2	
	CIK KIT Additive A	N107-A0025	250ul	
	CIK KIT Additive B	N107-B0025	250ul	
	CIK KIT Additive C	N107-C0025	250ul	

II. Instructionforusing:

Day 0 1)50ml of patient's whole blood was separated by lymphocyte separation solution, resulting in approximately 5×10^7 mononuclear cells.

2)Suspend cells in the 25ml CIK serum-free culture medium (N107-1000) with a cell concentration of approximately 1.5-2.0 × 10^6 cells/ml. Add additive A and incubate theculture flask at 37 °C in a 5% CO incubator.

Day 1 After 24 hours of culture, add additives B, C, and 1000IU/ml IL-2.

Day 3 Add 25ml CIK serum-free culture medium (containing 1000IU/ml IL- 2)

Day 5-6 On Day 5 or Day 6, add 50ml CIK serum-free culture medium (containing 1000IU/ml IL-2)

Day 7-14 According to the color change of the culture medium, add CIK serum-free culture medium (which can double the culture volume) daily, and 1000IU/ml IL-2 is also required to be added accordingly. Multiple culture bottles can be used for culture; It can also be added to the cultivation bag for culture.

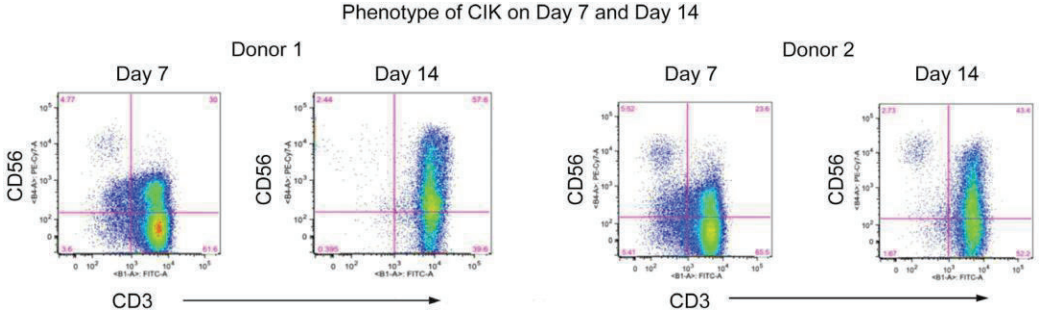
On day 10, detect the bacteria and fungi (culture negative); Trypan blue rejection test (live cells>95%). On day 13 or day 14, take out 1×10^5 cells for T cell subtype flow cytometry analysis, and cell suspensions shall be collected. After removing the supernatant by centrifugation, the cells shall be washed twice with physiological saline containing 2g/L albumin and 100IU/ml IL-2. Finally, suspend the cells in 400ml of physiological saline containing 20g/L albumin and 1000IU/ml IL-2.

III.The Following CIK Culture Data and Charts are for Reference Only (Complete Xeno-free Culture without the Addition of Autologous Plasma):

Culture Days	Day 0	Day 5	Day 7	Day 9	Day 11	Day13	Day 16
Donor 1	5×10^7	5.5×10^7	1.8×10^8	5.6×10^8	1.3×10^9	4.3×10^9	1.15×10^{10}
Donor 2	5×10^7	5.2×10^7	1.9×10^8	5.3×10^8	1.2×10^9	5.1×10^9	1.36×10^{10}

Note: Donor 1: peripheral blood mononuclear cell, cryopreserved at - 80 °Cfor 7 months, freezing medium (Haoke serum-free, protein-free freezing medium)

Donor 2: peripheral blood mononuclear cell, cryopreserved at - 80 °Cfor 3 months, with freezing medium (Haoke serum-free and protein-free freezing medium).



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