T cell Activation, human (91-01-0027)

[Product Description]

The T cell Activation reagent induces T cell activation and expansion in enriched T cells or PBMCs. The T cell Activation reagent is composed of biodegradable matrix-coated nanoparticles conjugated with mouse anti-human CD3 and mouse anti-human CD28 antibodies. The small sized particulate structure of the activation reagent enables its easy removal by media washing or centrifugation.

The recommended titer of 1:200 has been found to achieve efficient stimulation of majority of T cell subsets. For special applications, the optimal stimulation titer should be determined experimentally. Excessive amount of the T cell Activation reagent may result in over-activation of T cells risking activation-induced cell death. During cell expansion using the T cell Activation reagent, the cells take up to 13 days after reagent application to show optimal expansion. Restimulation after 13 days can be performed for longer cultivation. The T cell Activation reagent can be used together with cytokines such as human IL-2, IL-15, and IL-7 for more efficient activation.

[Product Specifications]

Cat. No.	Name	Size	Capacity
91-01-0027	T cell Activation, human	1mL	for up to 2×10^8 T cells

[Reactivity]

Human

[Product format]

Biodegradable matrix-coated nanoparticles conjugated with anti-CD3 and anti-CD28 antibodies supplied in phosphate buffered-saline (PBS), containing Human Serum Albumin (HSA), pH 7.0 - 7.4.

[Application]

T cell Activation reagent has been developed for in vitro activation and expansion of enriched T cell populations or cells from hematological cell populations (e.g. PBMCs).

[Storage]

Store at 2-8 ° C. Do not freeze.

[Materials required but not provided]

▲Human T cell culture media.

▲Human cytokines of choice, for example, IL-2, IL-15, IL-7.

[Protocol]

Notes:

▲All procedures are to be performed under sterile conditions.

▲After the initial T cell stimulation, the T cell Activation reagent must be allowed to interact with the culture for at least 2-3 days to reach the best T cell proliferation rate. Earlier removal of the activation reagent may reduce T cell proliferation.

▲The T cells should be transduced (for example, by using retro-viral or lenti-viral vectors) 1-2 days after activation by T cell Activation reagent. The optimal viral titer and timing of transduction varies among viral vectors, and may need to be determined experimentally.

▲ Residual EDTA can hamper T cell stimulation and needs to be removed prior to T cell stimulation.

1. Sample preparation

To prepare PBMC samples for T cell activation, PBMCs should be isolated by density gradient centrifugation, for example by using Ficoll.

2. T cell activation and expansion

This protocol has been optimized for gentle and efficient activation and expansion of enriched T cell populations using a titer of 1:200.

Purified T cells should be activated at an optimal surface density of 1×10^6 cells per cm^2 (Table 1). PBMCs should be activated at an optimal surface density of 2×10^6 per cm^2.

Culture plate	Growth area	Culture volume	T cell number	T cell Activation Reagent	T cell culture media
96 well	0.31 cm^2	0.2 mL	0.3×10^6	1 µL	199 µL
48 well	1 cm^2	1 mL	1×10^6	5 µL	995 µL
24 well	2 cm^2	2 mL	2×10^6	10 µL	1990 µL
12 well	4 cm^2	4 mL	4×10^6	20 µL	3980 µL
6 well	10 cm^2	5 mL	5×10^6	25 µL	4975 µL

Table 1: Optimal surface density when working with purified T cells.

Volumes given in Table 1 are for stimulation in 48-well plates. 5 µL of T cell Activation reagent is

effective for up to 1×10^6 purified T cells or up to 2×10^6 PBMCs in a total volume of 995 µ L T cell activation and expansion media. When working with fewer than 10^6 cells, continue using the volumes indicated in Table 1. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly. Irrespective of the culture vessel used, use 5 µL of T cell Activation reagent for activation of 1×10^6 T cells.

a. Activation in 48-well plate

1. Determine the cell number.

2. Resuspend the cells in appropriate volume of T cell culture media as indicated in Table 1.

3. Add appropriate volume of T cell Activation reagent as indicated in Table 1.

4. Incubate at 37° C, 5% CO_2 for up to 3 days.

5.Refresh T cell activation and expansion media 2–3 days after initial activation by adding new media or replacing the media using centrifugation:

i. Centrifuge the cell culture at 300×g for 10 minutes.

ii. Aspirate the supernatant completely.

iii. Add culture volume of fresh T cell activation and expansion media as indicated in Table 1.

iv. Incubate at 37° C, 5% CO2.

b. Expansion

1. Split the cell suspension every 2 days into two equal parts and add fresh T cell culture

media. Do not perform the first splitting earlier than 2 days after activation to ensure the best expansion result.

2. Incubate at 37° C, 5% CO_2.

3. On day 13, the cells are sufficiently expanded for downstream applications.

4. If needed, the time for expansion may be extended for up to 17 days depending on the cell state and experimental requirements.

For research and manufacturing use. Direct human use, including taking orally and injection are forbidden.