Cell Stimulation and Protein Transport Inhibitor Kit

Catalog No.: K083

Size: 50T/100T/200T

Kit components:

Reagents	50T	100T	200T	Storage
Cell Stimulation MIX Powder	50 μ g	50 μ g*2	50 μ g*4	-20°C , away from light
Cell Stimulation MIX Solvent	120 μ L	240 μ L	480 μ L	-20°C , away from light
Protein Transport Inhibitor MIX Powder	200μg	200μg*2	200 μ g*4	-20°C , away from light

Storage:

Store at -20℃ away from light for one year and -80℃ away from light for two years. After the dry powder is dissolved, it can be stored at -20°C away from light for 6 months , or it can be stored at -80°C away from light for 1 year after packaging.

Introduction:

Cell Stimulation and Protein Transport Inhibitor Kit is an optimized broad-spectrum immune cell stimulator and inhibitor that can induce and stimulate a variety of cells in vitro to produce cytokines and block the transport of secreted proteins to the extracellular.

Cell stimulation and Protein Transport Inhibitor Kit is mainly composed of Cell Stimulation MIX and Protein Transport Inhibitor MIX. Cell Stimulation MIX is a mixture of Phorbol 12-Myristate 13-Acetate (PMA) and Ionomycin, which can induce various cell activation and secrete cytokines.

Protein Transport Inhibitor MIX is mainly composed of Monensin and Brefeldin A, which can prevent the loss of cytokine transport. After cell membrane rupture, cytokines can be detected.

Reagent Preparation:

1) 500×Cell Stimulation MIX

Add 100 μL Cell Stimulation MIX Solvent to dissolve a vial of Cell Stimulation MIX Powder (50ug) and mix fully.

2) 1000×Protein Transport Inhibitor MIX

Add 50 μL absolute ethanol (self-prepared) to a vial of Protein Transport Inhibitor MIX Powder(200ug) and mix fully.

Note: Centrifuge at 2000~10000×g for several seconds before use and then open the cover for use. Absolute ethanol is volatile, please keep it sealed properly.

Assay Protocol:

Application 1: Cytokine content or activity detection in cell culture supernatant

1. Prepare the single cell suspension with complete medium (self-prepared), and adjust the cell density to 1~2×10^6/mL.

Note: The cell density should not be too high, and the maximum density should be less than 2×10^6/mL, high cell density will affect cell activation efficiency. Make sure the cells are in good condition before stimulation, especially for freshly prepared primary cells.

2. Add 2 μL of 500× Cell Stimulation MIX to each 1 mL of cell suspension, and incubate the cells at 37°C, 5% CO2 for 4~18 h (It is recommended to determine the optimal induction time by setting up a pre-experiment with different induction times for the cytokines to be tested. The common induction time can be refer to table 1).

3. Collect cell culture supernatant for the subsequent detection or store at -80°C for later use (the supernatant contains a variety of cytokines secreted by cells, which can be used to detect the content and activity of cytokines by ELISA or other biochemical reagents).

Application 2: Intracellular factor detection

1. Prepare the single cell suspension with complete medium (self-prepared), and adjust the cell density to 1~2×10^6/mL.

Note: The cell density should not be too high, and the maximum density should be less than 2×10^6/mL, high cell density will affect cell activation efficiency. Make sure the cells are in good condition before stimulation, especially for freshly prepared primary cells.

2. Add 2 μL of 500× Cell Stimulation MIX to each 1mL of cell suspension, and incubate the cells at 37°C, 5% CO2 for 0.5~1 h.

3. Add 1 μL of 1000×Protein Transport Inhibitor MIX to each 1mL of cell suspension, and incubate the cells at 37°C, 5% CO2 for 5~16 h (It is recommended to determine the optimal induction time by setting up a pre-experiment with different induction times for the cytokines to be tested. The common induction time can be refer to table 1).

4. Collect cell suspension, centrifuge at 200~300×g for 5 min, discard the supernatant and collect the cell pellet which could be used for subsequent intracellular factor detection after fixation.

Table 1: Reference of inducing condition of intracellular factors

Host	target cell	cytokines	Induction time
Mouse	Spleen T lymphocytes	IL-17A	5-6h
		IFN-γ	5-6h
		IL-4	5-6h
		IL-2	5-6h
		IL-10	5-6h
		IL-6	5-6h
Human	Peripheral blood T lymphocytes	IL-17A	5-6h
		IFN-γ	5-6h
		IL-4	5-6h
		IL-2	5-6h
		IL-10	5-6h
		IL-6	5-6h
		IL-21	5-6h

Note:

1.Due to the effect of Brefeldin A in Protein Transport Inhibitor MIX on CD69, it is recommended not to add Protein Transport Inhibitor MIX when detecting CD69. However, this operation may cause intracellular factors to be secreted outside the cell.

2.For your safety and health, please wear the lab coat and disposable gloves before the experiments.

3.This kit is for research use only.