Protein Transport Inhibitor MIX

Cat. No: E-CK-A013 **Size: 50 Tests/500 Tests**

Cat.	Products	50 Tests	500 Tests	Storage
E-CK-A013	Protein Transport Inhibitor MIX Powder (200 μg)	200 μg × 1 vial	200 μg × 10 vials	-20℃/-80℃, shading light
	Manual		One Copy	

Storage

1. Powder reagents can be stored for 1 year in the dark at -20°C and 2 years in the dark at -80°C.
2. The dissolved powder can be stored at -20°C for 6 months, or stored at -80°C for 1 year after subpackaged.

Introduction

Elabscience® Protein Transport Inhibitor MIX is mainly composed of Monensin and Brefeldin A, which can be used in combination with Cell Stimulation MIX [E-CK-A019] to prevent the loss of cytokine transport. After cell membrane rupture, cytokines can be detected. It can also be used alone to block protein transport from the Golgi apparatus and endoplasmic reticulum in cells.

Reagent Preparation

1000×Protein Transport Inhibitor MIX: Add 50 μL 33% DMSO solution (self-prepared) to a vial of Protein Transport Inhibitor MIX Powder (200 μg) and mix fully.

Note: Please centrifuge the powder at 8000~10000×g for 1 min, so that the powder will be gathered at the bottom of the tube before reagent preparation;

33% DMSO solution can be prepared by mixing 670 μL of sterile ultrapure water or sterile deionized water with 330 μL of anhydrous DMSO, then stored at -20 °C away from light.

Experimental Procedure

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 [E-CK-A019] to each 1mL of cell suspension, and incubate the cells at 37°C, 5%CO_2 for 1.5~1 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%CO_2 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).

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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

Species	Target cell	Cytokines/chemokines	Induction time
Mouse	Spleen T lymphocytes	IL-17A	5~6 h
		IFN-γ	5~6 h
		IL-4	5~6 h
		IL-2	5~6 h
		IL-10	5~6 h
		IL-6	5~6 h
Human	Peripheral blood T lymphocytes	IL-17A	5~6 h
		IFN-γ	5~6 h
		IL-4	5~6 h
		IL-2	5~6 h
		IL-6	5~6 h
		IL-10	5~6 h
		IL-21	5~6 h

Troubleshooting

Symptoms	Causes	Comments
Cytokines were detected in supernatants but not in cells	The incubation time of 1000×Protein Transport Inhibitor MIX is insufficient.	Appropriately increase the incubation time of 1000×Protein Transport Inhibitor MIX.
More cell loss	Centrifugal conditions are not appropriate.	Unfixed living cells centrifugal force is less than 300×g, the speed of acceleration is less than 3,the speed of deceleration is less than 2, which can greatly reduce the cell loss caused by centrifugation.
	Too many cells, inadequate fixation.	Increase fixed liquid volume and extend fixation time.

Cautions

1. This kit is for research use only.
2. 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.
3. Please take safety precautions and follow the procedures of laboratory reagent operation.
4. Please store the product at the appropriate temperature to avoid failure.

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