Cell Stimulation MIX Kit

Cat. No: E-CK-A019 **Size: 50 Assays/200 Assays/500 Assays**

Cat.	Products	50 Assays	200 Assays	500 Assays	Storage
E-CK-A011	Cell Stimulation MIX Powder (50 μg)	50 μg × 1 vial	50 μg× 4 vials	50 μg × 10 vials	-20℃/-80℃, shading light
E-CK-A012	Cell Stimulation MIX Solvent	120 μL	480 μL	1200 μL	-20°C, 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® Cell Stimulation MIX Kit is a mixture of Phorbol 12-Myristate 13-Acetate (PMA) and Ionomycin, which can induce various cell activation and secrete cytokines. The activated cell supernatant can be used to detect cytokines by immunological methods. It is possible to add Protein Transport Inhibitor MIX [E-CK-A013] at an appropriate time to detect cytokines by flow cytometry.

Reagent Preparation

500×Cell Stimulation MIX: Add 100 μL Cell Stimulation MIX Solvent to dissolve a vial of Cell Stimulation MIX Powder (50 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.

Experimental Procedure

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%CO_2 for 4~18 h (It is recommended to determine the optimal induction time by setting up a

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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%CO_2 for 1.5~1 h.
3. Add 1 μL of 1000×Protein Transport Inhibitor MIX [E-CK-A013] 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).
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
No cytokines detected	The cell density is too large.	Adjust cell density to 1~2×10^6/mL.
	Red blood cell interference.	Tissue containing more red blood cells should be treated with red blood cell lysate first.
	The reagent failed.	Preserve the reagent reasonably and use within the validity period.
	The antibody effect is not good.	Use effective antibody as positive control.
	The effect of cell fixation and permeabilization is not good.	Use effective fixative and permeabilization buffer.
	The induction time is not enough.	Set the induction time gradient to select the best induction time.
Overexpression of intracellular factors	Poor cell state and more dead cells.	Ensure that the cells are in good condition before induction, and eliminate the interference of dead cells.
	Non-specific binding of antibodies.	Increase antibody blocking process to reduce non-specific binding.
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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