

## Cell Stimulation and Protein Transport Inhibitor Kit

Cat. No: E-CK-A091

Size: 50 Assays/100 Assays/200 Assays/500 Assays

| Cat.      | Products  | 50 Assays       | 100 Assays       | 200 Assays       | 500 Assays        | Storage               |
|-----------|---|-----------------|------------------|------------------|-------------------|-----------------------|
| E-CK-A011 | Cell Stimulation MIX Powder ( 50 µg )             | 50 µg × 1 vial  | 50 µg × 2 vials  | 50 µg × 4 vials  | 50 µg × 10 vials  | -20 °C, shading light |
| E-CK-A012 | Cell Stimulation MIX Solvent                      | 120 µL          | 240 µL           | 480 µL           | 1200 µL           | -20 °C, shading light |
| E-CK-A013 | Protein Transport Inhibitor MIX Powder ( 200 µg ) | 200 µg × 1 vial | 200 µg × 2 vials | 200 µg × 4 vials | 200 µg × 10 vials | -20 °C, shading light |
|           | Manual  |                 |                  | One Copy         |                   |                       |

### Storage

1. Powder reagents (E-CK-A011 and E-CK-A013) 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 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 ( 50 µg ) 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 ( 200 µg ) 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.**

### For Research Use Only

## 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  $1\sim 2\times 10^6/\text{mL}$ .

**Note:** The cell density should not be too high, and the maximum density should be less than  $2\times 10^6/\text{mL}$ , 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  $\mu\text{L}$  of 500 $\times$  Cell Stimulation MIX to each 1 mL of cell suspension, and incubate the cells at 37  $^{\circ}\text{C}$ , 5% $\text{CO}_2$  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  $^{\circ}\text{C}$  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  $1\sim 2\times 10^6/\text{mL}$ .

**Note:** The cell density should not be too high, and the maximum density should be less than  $2\times 10^6/\text{mL}$ , 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  $\mu\text{L}$  of 500 $\times$  Cell Stimulation MIX to each 1mL of cell suspension, and incubate the cells at 37  $^{\circ}\text{C}$ , 5% $\text{CO}_2$  for 1.5~1 h.
3. Add 1  $\mu\text{L}$  of 1000 $\times$ Protein Transport Inhibitor MIX to each 1mL of cell suspension, and incubate the cells at 37  $^{\circ}\text{C}$ , 5% $\text{CO}_2$  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 $\times g$  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- $\gamma$        | 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- $\gamma$        | 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  |
|---|---|---|
| <b>No cytokines detected</b>                                    | The cell density is too large.  | Adjust cell density to $1\sim 2 \times 10^6/\text{mL}$ .  |
|   | 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.  |
| <b>Overexpression of intracellular factors</b>                  | 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.  |
| <b>Cytokines were detected in supernatants but not in cells</b> | The incubation time of 1000 $\times$ Protein Transport Inhibitor MIX is insufficient. | Appropriately increase the incubation time of 1000 $\times$ Protein Transport Inhibitor MIX.                |

|                       |   |   |
|-----------------------|---|---|
| <b>More cell loss</b> | 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.