TECHNICAL MANUAL

Cell Stimulation and Protein Transport Inhibitor Kit

- **SKU CODE:** AKES084
- **SIZE:** 50 Assays / 100 Assays / 200 Assays
- **DETECTION PRINCIPLE:** Cell Stimulation & Protein Transport Inhibition
- **RUO:** Research-Use-Only

Revised: 10/26

Cell Stimulation and Protein Transport Inhibitor Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Specification:

50 Assays / 100 Assays / 200 Assays

Main components:

Cell Stimulation MIX (PMA and Ionomycin); Protein Transport Inhibitor MIX (Monensin and Brefeldin A)

Applications:

Cytokine content or activity detection in cell culture supernatant; intracellular factor detection

Sample type:

Single cell suspensions (e.g. mouse spleen T lymphocytes, human peripheral blood T lymphocytes)

2. Storage & Expiry

This product should be stored at -20°C or -80°C in dark conditions. Powder reagents can be stored for up to 12 months at -20°C or 24 months at -80°C. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

Once dissolved, the powder reagents can be stored at -20°C for 6 months, or at -80°C for 1 year after aliquoting.

3. Product Description

The 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 space.

The 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 cytokine secretion.

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

4. Kit Contents

No	Component Name	50 Assays	100 Assays	200 Assays	Storage
1	Cell Stimulation MIX Powder (50 µg)	50 µg × 1 vial	50 µg × 2 vials	50 µg × 4 vials	-20°C/-80°C, protect from light
2	Cell Stimulation MIX Solvent	120 µL	120 µL × 2 vials	120 µL × 4 vials	-20°C, protect from light
3	Protein Transport Inhibitor MIX Powder (200 µg)	200 µg × 1 vial	200 µg × 2 vials	200 µg × 4 vials	-20°C/-80°C, protect from light
4	Manual	One copy			

Note: *All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.*

Additional materials required:

1. 37°C, 5% CO_2 incubator.
2. Complete cell culture medium (self-prepared).
3. Anhydrous DMSO and sterile ultrapure or deionized water (for preparing 33% DMSO solution).
4. Centrifuge.
5. Precision pipettes and disposable pipette tips.

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. 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 may cause intracellular factors to be secreted outside the cell.
5. Please store the product at the appropriate temperature to avoid reagent failure.
6. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
7. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. Reagent Preparation

1. **500× Cell Stimulation MIX:** Add 100 µL Cell Stimulation MIX Solvent to dissolve one vial of Cell Stimulation MIX Powder (50 µg) and mix fully.
2. **1000× Protein Transport Inhibitor MIX:** Add 50 µL 33% DMSO solution (self-prepared) to one 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 is 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.

7. Assay Procedure

A. 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 pre-experiment with different induction times for the cytokines to be tested; for common induction times, refer to Table 1 in Section 8).
3. Collect the cell culture supernatant for 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).

B. 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 1 mL 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 1 mL 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; for common induction times, refer to Table 1 in Section 8).
4. Collect the cell suspension, centrifuge at 200~300×g for 5 min, discard the supernatant and collect the cell pellet, which can be used for subsequent intracellular factor detection after fixation.

8. Reference Induction Conditions

Table 1: Reference inducing conditions 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

9. Troubleshooting

Symptoms	Causes	Comments
No cytokines detected	The cell density is too high.	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 has failed.	Store the reagent appropriately and use within the validity period.
	The antibody performance is poor.	Use an effective antibody as a positive control.
	Cell fixation and permeabilization are not effective.	Use an effective fixative and permeabilization buffer.
	The induction time is not long enough.	Set an 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.	Add an antibody blocking step to reduce non-specific binding.
Cytokines 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	Centrifugation conditions are not appropriate.	For unfixed living cells, a centrifugal force of less than 300×g, an acceleration speed of less than 3 and a deceleration speed of less than 2 can greatly reduce cell loss caused by centrifugation.
	Too many cells, inadequate fixation.	Increase the fixation solution volume and extend the fixation time.

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.