

Human Granzyme B Activity Detection Substrate for Flow Cytometry

Cat. No: E-CK-A480

Size: 20 Tests/100 Tests

Cat.	Products	20 Tests	100 Tests	Storage
E-CK-A480	Human Granzyme B Substrate(Green)(1mM)	20 µL	100 µL	-20°C, shading light
	Manual		One Copy	

Storage

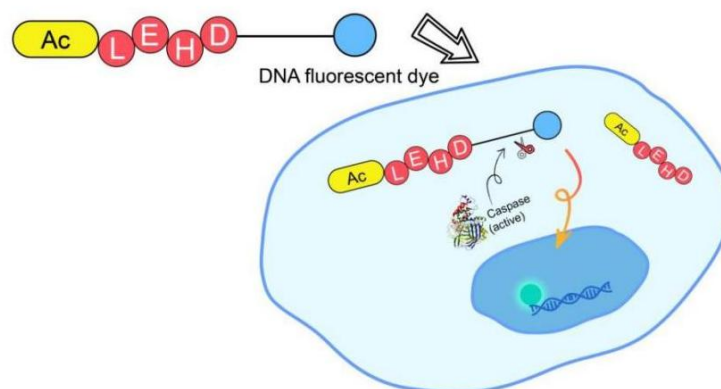
The Human Granzyme B Substrate(Green)(1mM) should be stored at -20°C away from light and is valid for 1 year.

Introduction

Elabscience® Human Granzyme B Substrate(Green) is cell-membrane-permeable green fluorescent substrate. It can be used to detect Granzyme B activity in living cells without affecting cellular physiological functions or Granzyme B activity. After substrate staining, cells with Granzyme B activity exhibit green fluorescence, which can be detected by using the FL1 or FITC channel of a flow cytometer.

Detection Principle

Human Granzyme B Substrate (Green) is generated by conjugating a Granzyme B-specific recognition peptide (sequence IEPD) with a high-affinity DNA fluorescent dye. The substrate is cell-membrane-permeable and can penetrate the plasma membrane into the cytoplasm. In living cells without active Granzyme B, charge repulsion occurs between the substrate and DNA, resulting in no cellular fluorescence signal. In contrast, in living cells with active Granzyme B, the activated Granzyme B cleaves the substrate and releases the high-affinity DNA dye. The released DNA dye binds to intracellular DNA and emits green fluorescence, thereby enabling the detection of Granzyme B activity.



For Research Use Only

Detection Sample Types

Suspension Cells

Materials Not Supplied

1) Reagents

sterile PBS buffer

2) Instruments

Centrifuge, CO₂ incubator, flow cytometer, biosafety cabinet

3) Consumable materials

Petri dishes, pipette, 6-well plates

Experimental Protocol

➤ For flow cytometry

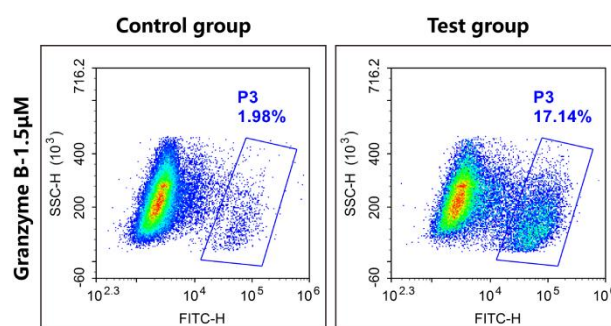
- (1) Collect the cells by centrifuging at 250 g for 5 min, discard the supernatant. Resuspend the cell pellet with 1 mL PBS buffer, centrifuge at 250 g for 5 min, and discard the supernatant.
- (2) Resuspend 2–5×10⁵ cells per group in 400 μL PBS buffer. Add 1 μL Human Granzyme B Substrate(Green) and immediately mix fully (The final concentration of Granzyme B Substrate is 2.5 μM. Incubate cells at 37°C for 30 min with shading light.

Note: It is recommended to use PBS buffer without serum or BSA for incubation with Human Granzyme B Substrate (Green), as serum and BSA can increase non-specific background fluorescence.

If the Granzyme B activity in cells is relatively low, the dosage of Human Granzyme B Substrate (Green) (1 mM) can be increased to achieve a final concentration of 3–5 μM

- (3) After incubation, collect the cells from each group into 2 mL tubes respectively. Add 1 mL PBS to resuspend the cells, centrifuge at 250 g for 5 min, and discard the supernatant.
- (4) Resuspend the cell pellet in an appropriate volume of PBS buffer and detect samples by flow cytometer with FL1 or FITC channel.

Typical Results



Control group: Jurkat cells were cultured without drug administration;
Test group: Jurkat cells were cultured with 1×Cell Stimulation MIX (E-CK-A011) for 5h.

Figure 1: Jurkat cells were stained with 2.5 μm Human Granzyme B Substrate (Green) and analyzed by flow cytometry.

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Cautions

1. This product is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Human Granzyme B Substrate(Green)(1mM) is recommended to aliquot to smaller quantities and use out within 6 months. Avoid repeated freezing and thawing.
4. This product can be used to detect Granzyme B enzyme activity in living cells, but not applicable to fixed cells.
5. Excessively high acceleration and deceleration speeds of the centrifuge may cause loss of cell quantity and viability. It is suggested to adjust the acceleration no more than 3 and deceleration no more than 2, that is, $Acc \leq 3$, $Dec \leq 2$.

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