STYX™ Violet Fixable Viability Kit

Cat. No: E-CK-A167 **Size: 50 Tests/100 Tests/200 Tests**

Cat. No	Product	50 Tests	100 Tests	200 Tests	Storage
E-CK-A167A	STYX™ Violet Fixable Viability Dye, Powder	Powder×1 vial	Powder×2 vials	Powder×4 vials	≤-20°C,desiccate, shading light
E-CK-A166B	DMSO, anhydrous	60 μL	120 μL	240 μL	≤-20°C,desiccate, shading light
	Manual	1 copy			

Storage

STYX™ Violet Fixable Viability Dye, Powder is stable for 1 year when stored at ≤ -20°C. After reconstitution in DMSO, it is recommended to aliquot the solution, store at ≤ -20°C protected from light and moisture, avoid repeated freeze-thaw cycles, and use within one month.

Detection Principle

STYX™ Violet Fixable Viability Dye is a membrane-impermeant, amine-reactive fluorescent dye that irreversibly binds to free amines on the cell surface and and inside cells. In viable cells, staining is restricted to the surface. When the cell membrane is compromised, the dye enters and binds intracellular amines, resulting in significantly stronger fluorescence in dead cells. This enables clear distinction between live and dead cells. The staining remains stable after fixation, permeabilization, and cryopreservation.

STYX™ Violet Fixable Viability Dye has optimal excitation and emission maxima at approximately 416 nm and 451 nm, respectively, and can be detected using the Pacific Blue detection channel of a flow cytometer.

Reagents and Materials Not Supplied

1. **Reagents:**

   PBS, Cell Fixation Buffer, Cell Permeabilization Buffer

2. **Materials:**

   1.5 mL EP tube, 2 mL EP tube

3. **Instrument:**

   Centrifuge, Flow Cytometer

Experimental Operation

➢ Reagent Preparation

Preparation of STYX™ Violet Fixable Viability Dye Working Solution: Bring one vial of the STYX™ Violet Fixable Viability Dye powder and the vial of DMSO to room temperature before removing the caps. One vial of STYX™ Violet Fixable Viability Dye powder, centrifuge at 8000-10000×g for 1 minute to settle the powder to the bottom of the vial. Add 50 µL of DMSO to the vial and mix well until the powder is completely dissolved. It is recommended to aliquot the reconstituted dye, store at ≤ -20°C protected from light and moisture, avoid repeated freeze-thaw cycles.

➢ Staining Procedure

(1) Harvest cells and centrifuge at 300×g for 5 minutes. Discard the supernatant. Wash the cells with 1 mL of PBS, centrifuge at 300×g for 5 minutes, and then discard the supernatant.

(2) Resuspend the cells in 1 mL of PBS, count the cells, and adjust the cell density to 1×10^6 cells /mL with PBS. Take 1mL of cell suspension per tube, add 1 µL of STYX™ Violet Fixable Viability Dye working solution, mix gently and incubate for 30 minutes at room temperature or 4℃, protected from light.

Note: PBS is recommended for this step. Tris buffer and solutions containing sodium azide or extraneous proteins cannot be used as cell staining buffers to wash or resuspend cells to avoid interfering with amine-reactive fluorescent dye staining. Proteins can be contained in the buffers in subsequent steps.

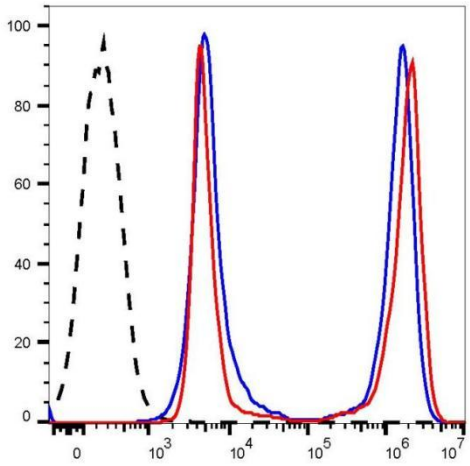
(3) Centrifuge at 300×g for 5 minutes and discard the supernatant. Resuspend the cells in 1 mL of PBS, centrifuging again, and discarding the supernatant.

(4) Resuspend the cells in 100 µL of Cell Staining Buffer [E-CK-A107]. Stain cell surface markers as need.

(5) After incubating with the cell surface marker, add 1 mL of Cell Staining Buffer, centrifuge at 300×g for 5 min, discard the supernatant. Resuspend the cells in an appropriate volume of PBS or Cell Staining Buffer for flow cytometric analysis.

Note: For intracellular or nuclear marker staining requiring fixation and permeabilization, please use the Intracellular Fixation/Permeabilization Buffer Kit [E-CK-A109] or Foxp3/Transcription Factor Staining Kit [E-CK-A108] according to their respective instructions.

Typical data



Heat-killed (mixed 1:1 with live) C57BL/6 mouse splenocytes were stained with STYX™ Violet Fixable Viability Dye followed by fixation and permeabilization using the Intracellular Fixation/Permeabilization Buffer Kit [E-CK-A109] and analyzed before fixation (blue) or after fixation (red). Cells alone, without STYX™ Violet Fixable Viability Dye staining, are indicated in black.

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Store the product under the recommended conditions..
4. DMSO is hazardous; avoid contact with skin and eyes.
5. DMSO will freeze at -20°C. Thaw at room temperature before opening. Do not heat. After opening, avoid repeated freeze-thaw cycles.