ab112130

Generic Caspase Activity Assay Kit – Fluorometric Green

Instructions for Use

For detecting Caspase activity in cells by using our proprietary green fluorescence probe.

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Abcam's Activity Assay kits are a set of tools for monitoring cellular functions. The activation of caspase is widely accepted as a reliable indicator for cell apoptosis. Most caspases have substrate selectivity for the peptide sequence Val-Ala-Asp (VAD). ab112130 uses TF2-VAD-FMK as a fluorescent indicator for most caspase activities. The cell permeable and nontoxic TF2-VAD-FMK irreversibly binds to activated caspase-1, -3, -4, -5, -6, -7, -8 and -9 in apoptotic cells. Once bound to caspases, the fluorescent reagent is retained inside the cell. The binding event prevents the caspases from further catalysis but will not stop apoptosis from proceeding. Within 15 minutes incubation, it starts to react with active caspase enzymes.

ab112130 provides all the essential components with an optimized assay protocol. It is designed to detect cell apoptosis by measuring generic activation of caspases (caspase-1, -3, -4, -5, -6, -7, -8 and -9) in live cells.

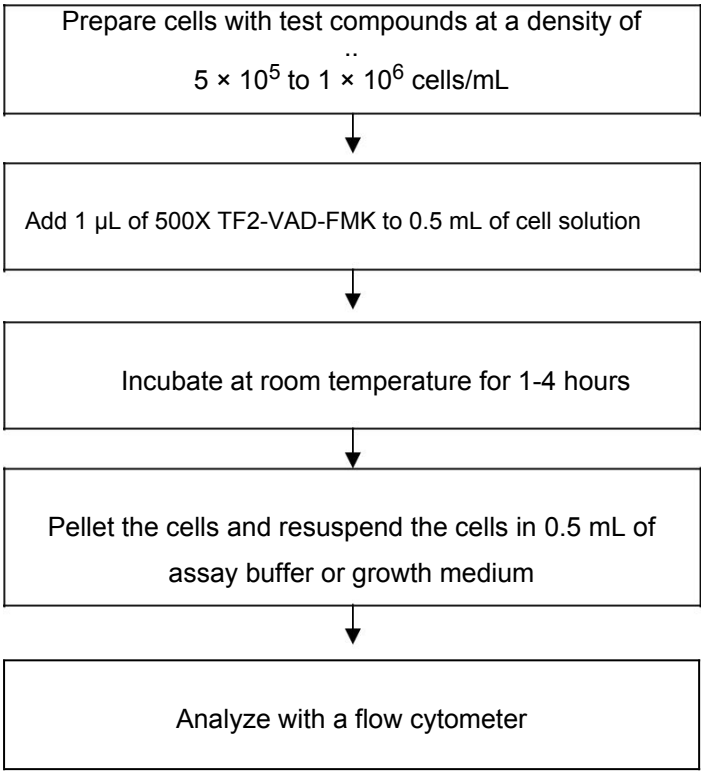
ab112130 is used for either the quantification of most activated caspase activities in apoptotic cells or screening of caspase inhibitors. TF2-VAD-FMK, the green label reagent, allows for direct detection of activated caspases in apoptotic cells by a flow cytometer at Ex/Em = 488/520 nm.

Kit Key Features

- **Convenient and Robust:** Formulated to have minimal hands-on time.
- **Non-Radioactive:** No special requirements for waste treatment.
- **Optimized Performance**: Provide optimal conditions for the detection of many caspase activities.
- **Enhanced Value**: Less expensive than the sum of individual components.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: 500X TF2-VAD-FMK	1 x100 µL
Component B: Assay Buffer	1 x 50 mL
Component C: 500X Propidium Iodide	1 x100 µL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Assay Protocol

Note: This protocol is for each Sample.

A. For each sample, prepare cells in 0.5 mL warm medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

B. Treat cells with test compounds for a desired period of time to induce apoptosis, and create positive and negative controls.

C. Add 1 µL of 500X TF2-VAD-FMK (Component A) into the treated cells (from Step 2), and incubate the cells in a 37°C, 5% CO_2 incubator for 1-4 hours.

Note 1: For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with TF2-VAD-FMK.

Note 2: The appropriate incubation time depends on the individual cell type and the cell concentration used. Optimize the incubation time for each experiment.

D. Wash and spin the cells twice. Resuspend the cells in 0.5 mL of assay buffer or growth medium.

Note: TF2-VAD-FMK is fluorescent, thus it is important to wash out any unbound reagent to remove the background.

E. If desired, label the cells with a DNA stain (such as propidium iodide or 7-AAD for dead cells).

F. If desired, fix cells.

G. Monitor the fluorescence intensity with a flow cytometer using the FL1 channel (Ex/Em = 490/525 nm). Gate on the cells of interest, excluding debris.

6. Data Analysis

In live non-apoptotic cells, TF2-VAD-FMK detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, TF2-VAD-FMK binds to active caspases resulted in increased staining intensity.

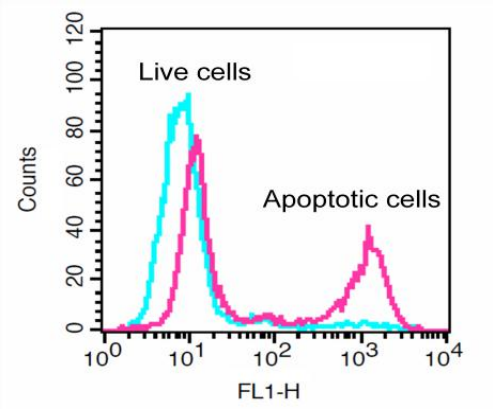


Figure 1. The increase in TF2-VAD-FMK fluorescence intensity with the addition of Camptothecin in Jurkat cells. Jurkat cells were treated without (Blue) or with 20 µM camptothecin (Red) in a 37^{o}C, 5% CO_2 incubator for 4-5 hours, and then dye loaded with TF2-VAD-FMK for 1 hour.

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "contact us" on www.abcam.com for the phone number for your region).

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