



ab112131

Generic Caspase Activity Assay Kit – Fluorometric Red

Instructions for Use

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

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



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Table of Contents

1. Introduction	3
2. Protocol Summary	5
3. Kit Contents	6
4. Storage and Handling	6
5. Assay Protocol	7
6. Data Analysis	9
7. Troubleshooting	10

1. Introduction

Abcam 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).

ab112131 is designed to monitor cell apoptosis through measuring generic caspases (caspase-1, -3, -4, -5, -6, -7, -8 and -9) activation in live cells with our red fluorescent TF5-VAD-FMK probe. Most caspases have substrate selectivity for the peptide sequence Val-Ala-Asp (VAD). The cell permeable and nontoxic TF5-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 activation but will not stop apoptosis from proceeding.

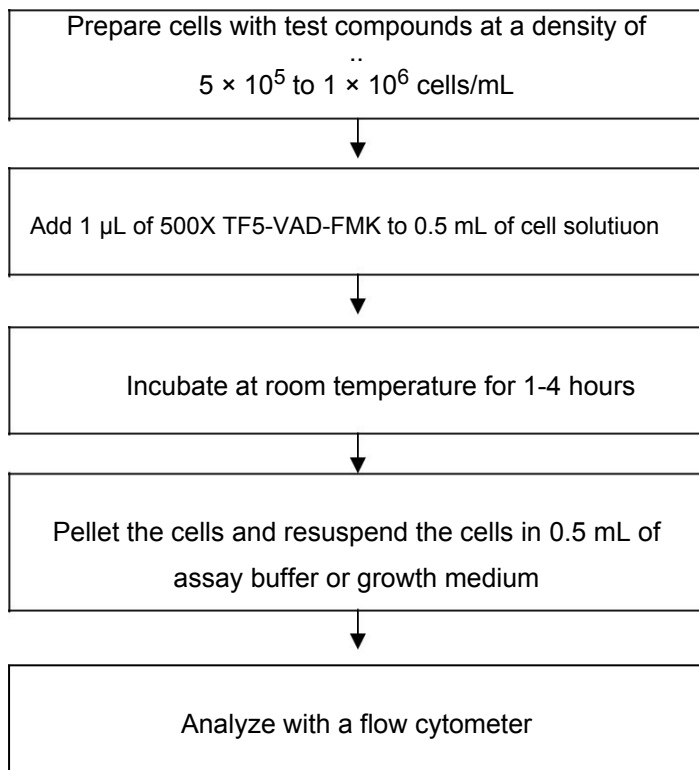
ab112131 can be conveniently used with the common fluorescence instruments equipped with the light sources and filters Ex/Em = ~647/660 nm. The red label allows for direct detection of activated caspases in apoptotic cells by a flow cytometer. It can be used for the quantification of most activated caspases activities in apoptotic cells, or for the screening of caspases inhibitors. The kit provides all the essential components.

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 Sample



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

3. Kit Contents

Components	Amount
Component A: 500X TF5-VAD-FMK	1 x100 µL
Component B: Assay Buffer	1 x 50 mL
Component C: 500X Green-DCS1	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 μ L of 500X TF5-VAD-FMK (Component A) into the treated cells (from Step B), and incubate the cells in a 37°C, 5% CO₂ 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 TF5-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.

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

Note: TF5-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 Green-DCS for dead cells, which can be detected in the FL1 channel (Ex/Em = 490/525 nm)].
- F. If desired, fix cells.
- G. Monitor the fluorescence intensity with a flow cytometer using the FL4 channel (Ex/Em = 650/670 nm). Gate on the cells of interest, excluding debris.

6. Data Analysis

In live non-apoptotic cells, TF5-VAD-FMK detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, TF5-VAD-FMK binds to active caspases resulted in increased staining intensity.

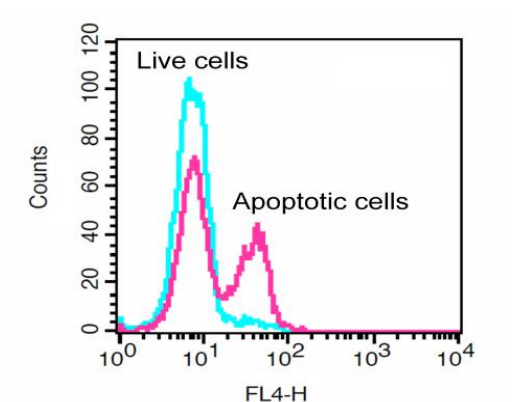


Figure 1. The increase in TF5-VAD-FMK fluorescence intensity with the addition of Camptothecin in Jurkat cells. Jurkat cells were treated without (Blue) or with 20 μ M camptothecin (Red) in a 37°C, 5% CO₂ incubator for 4-5 hours, and then dye loaded with TF5-VAD-FMK for 1 hour.

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 sample types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

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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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