ELISA PRODUCT INFORMATION & MANUAL

Caspase-8 Assay Kit (Fluorometric)

NBP2-54870

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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1. Overview

Caspase-8 Assay Kit (Fluorometric) (NBP2-54870) provides a convenient means for detecting activated caspase-8 in living cells. The assay utilizes a caspase-8 inhibitor IETD-FMK conjugated to sulfo-hodamine (Red-IETD-FMK) as the fluorescent marker. Red-IETD-FMK is cell permeable, nontoxic, and irreversibly binds to activated caspase-8 in apoptotic cells. The fluorescence label allows detection of activated caspase-8 in apoptotic cells directly by fluorescence microscopy, flow cytometry, or fluorescence plate reader.

2. Protocol Summary

Induce apoptosis or treat cells using desired method. Culture and negative control cells in parallel.

Aliquot 300 µL of each induced and controls into tubes.

Add 1 µL of Red-IETD-FMK into each tube.

Incubate for 0.5-1 hour at 37°C, with 5% CO2.

Centrifuge for 5 minute at 3,000 rpm and remove supernatant.

Resuspend cells in 0.5 ml of Wash Buffer.

Centrifuge for 5 minute at 3,000 rpm and remove supernatant.

Resuspend with Wash Buffer, adding volume accordingly with Method of analysis:

Quantification by Flow Cytometry (300 µL);

Detection by Fluorescence Microscopy or Plate Reader (100 µL).

3. Precautions

Please read these instructions carefully prior to beginning the Staining.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Staining kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
Red-IETD-FMK	100 µL	-20°C
Wash Buffer	2 x 100 mL	-20°C
Z-VAD-FMK	10 µL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this Staining:

- Centrifuge tubes
- Fluorescence plate reader measuring fluorescence intensity at Ex/Em = 540/570 nm.

  ΔNote: 488/570 nm will also work, although it's not an optimal wavelength
- Flow cytometer
- Fluorescence Microscopy
- Cell line of choice
- Reagents for induction of apoptosis
- Black microtiter plate
- Microslide and coverslip

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single Staining well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the Staining.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Reagents are supplied ready to use.

10. Staining Procedure

Thaw all reagents thoroughly and mix gently.

10.1 Staining Protocol:

10.1.1 Induce apoptosis in cells (1 x 10^6 cells/ml) by desired method. Concurrently incubate a control culture without induction.

10.1.2 Negative control: Can be prepared by adding Z-VAD-FMK at 1 µL/mL to an induced culture.

10.1.3 Aliquot 300 µL of each induced and control cultures into tubes.

10.1.4 Add 1 µl of Red-IETD-FMK into each tube.

10.1.5 Incubate for 0.5-1 hour at 37°C incubator with 5% CO2.

10.1.6 Centrifuge cells at 3000 rpm for 5 minutes and remove supernatant.

10.1.7 Resuspend cells in 0.5 ml of Wash Buffer.

10.1.8 Repeat step 11.1.5.

10.1.9 Next steps depend on methods of analysis:

10.2 Quantification by Flow Cytometry

Resuspend cells in 300 µl of Wash buffer and put samples on ice.

Analyzed samples by flow cytometry using the FL-2 channel.

10.3 Detection by Fluorescence Microscopy

Resuspend cells in 100 µl Wash buffer.

Put one drop of the cell suspension onto a microslide and cover with a coverslip.

Observe cells under a fluorescence microscope using rhodamine filter.

10.4 Analysis by Fluorescence Plate Reader

Resuspend cells in 100 µl Wash Buffer.

Transfer the cell suspension to each well of the black microtiter plate.

Measure the fluorescence intensity at Ex/Em = 540/570 nm.

ΔNote: Ex/Em=488/570 nm will also work, although it's not an optimal wavelength.

11. Calculations

Fold-increase in Red Caspase-8 activity can be determined by comparing the results of treated samples with the level of the untreated control.

Background reading from cell lysates and buffers should be subtracted from the readings of both treated and the untreated samples before calculating fold increase in Red Caspase-8 activity.

For detection by Fluorescence Microscopy, Caspase-8 positive cells appear to have brighter red signals, whereas caspase-8 negative control cells show much weaker signal.

12. Notes