KAMIYA BIOMEDICAL COMPANY

Apoptosis / Caspase Assay Kit

For the assay and inhibition of caspase activities associated with apoptosis

Cat. No. AC-050

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

Apoptosis / Caspase Assay Kit

Cat. No. AC-050

PRODUCT

The K-ASSAY® Apoptosis / Caspase Assay Detection Kit provides caspase substrates and inhibitors that offer several options for the detection of apoptosis. The peptide-AFC substrates permit the assay of cell extracts to detect and quantify caspase activities associated with apoptosis. Substrates for caspase-1 (Ac-YVAD-AFC) and caspase-3 (Ac-DEVD-AFC) are provided. The release of free AFC from the substrates can be measured either fluorometrically (ex: 400 nm; em: 505 nm) or spectrophotometrically (380 nm). The Apoptosis / Caspase Assay Detection Kit also provides cell permeable inhibitors of caspases which can be used to block apoptosis. When applied to cells, Z-VAD-FMK inhibits all known caspase activities associated with apoptosis. Z-DEVD-FMK is an inhibitor of caspase-3. In addition, the kit provides an AFC standard for use with the fluorescent substrates, and an inhibitor negative control. For research use only, not for use in diagnostic procedures.

COMPONENTS

A. **Ac-YVAD-AFC** [Ac-Tyr-Val-Ala-Asp-7-Amino-4-Trifluoromethyl Coumarin], TFA salt, MW 719
3 mg
Fluorogenic / Chromogenic substrate to detect the activity of caspase-1
References: 2,11,13,15,22

B. **Ac-DEVD-AFC** [Ac-Asp-Glu-Val-Asp-7-Amino-4-Trifluoromethyl Coumarin], TFA salt, MW 729
3 mg
Fluorometric / chromogenic substrate to detect the activity of caspase-3.
References 4, 9, 10, 12, 13, 14, 17, 20, 21

C. **AFC** [7-Amino-4-Trifluoromethyl Coumarin] MW 229
3 mg

D. **Z-VAD-FMK** [Z-Val-Ala-Asp(OMe)-Fluoromethyl Ketone], TFA salt, MW 468
1 mg
A cell permeable inhibitor of caspase-1 that blocks the activation of downstream caspases
References: 3,4,6,9,10,16,18,20

E. **Z-DEVD-FMK** [Z-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-Fluoromethyl Ketone], TFA salt, MW 668
1 mg
A specific inhibitor of caspase-3/ CPP-32
References: 1,5.7,8,10,13,19, 20, 21

F. **Z-FA-FMK** [Z-Phe-Ala-Fluoromethyl Ketone], TFA salt, MW 386
1 mg
A negative control lacking aspartic acid at the P1 position.
References6, 16, 18, 19

Materials or equipment required but not provided

1. DMSO for dissolving reagents
2. Assay buffer. The recommended buffer is 0.1M HEPES (pH 7.4), 2mM DTT, 0.1% CHAPS, 1% sucrose.

PROTOCOLS

Reagent preparation and use:
A. Ac-Tyr-·Val-Ala-Asp-AFC; M.W. 720;

1. This vial contains 3 mg of product. Dilute in 208 µl of dry DMSO, giving you a 20mM solution. This solution should be used as a stock, Store in –20°C freezer.

2. Buffer: 0.1M HEPES (pH 7.4), 2mM DTT, 0.1% CHAPS, 1% sucrose.

3. Mix buffer and a 50 µl aliquots of cell lysates to a volume of 495 µl. Add 5 µl of substrate stock

4. Incubate at R.T. for 30 minutes.

5. Take T_0 measurements and monitor release of free AFC using fluorometer settings of 400 nm excitation and 505 nm emission. When a significant amount of fluorescence has been generated, record $T_{end.}$ Calculate the enzyme kinetics from a recently plotted standard curve (See Standard AFC below).

B. Ac-Asp-Glu-Val-Asp-AFC; M.W. 729;

1. This vial contains 3mg of product. Dilute in 206 µl of dry DMSO, giving you a 20mM solution. This is your stock solution. Store in –20°C freezer

2. Recommended buffer: 0.1M HEPES (pH 7.4), 2mM DTT, 0.1% CHAPS, 1% sucrose.

3. Mix buffer and a 50 µl aliquots of cell lystes to a volume of 495 µl. Add 5 µl of substrate stock.

4. Incubate at room temperature far 30 minutes

5. Take T_0 measurements and monitor release of free AFC using fluorometer settings of 400 nm excitation and 505 nm emission. When a significant amount of fluorescence has been generated, record $T_{end.}$ Calculate the enzyme kinetics from a recently plotted standard curve (See "AFC" below).

C. AFC (7-amino-4-trifluoromethyl Coumarin); M.W. 229;

1. Dissolve 3 mg of free AFC in 16.23 ml DMSO for a stock solution. Store in a –20°C freezer.

2. Dilute stock solution1:10 for a working solution.

3. Prepare free AFC standards as follows, and record fluorescence:

µl buffer	µl AFC working solution
500	0
490	10
480	20
470	30

4. Graph µmoles of free AFC on the X-axis, and fluorescent units observed on the Y-axis.

5. Use the slope of the standard curve to convert fluorescence units generated by enzyme to activity.

D. Z-Val-Ala-Asp(OMe)-FMK; M.W. 468;

1. This vial contains 1 mg of product. Dilute in 107 µl of dry DMSO, giving a 20mM stock solution. Store in –20℃ freezer.

2. Add 2ul of stock solution to 1 ml of culture medium to get 40 µM final Z-VAD-FMK concentration ($1x10^6$ cell count)

E. Z-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)-FMK; M.W. 668;

1. This vial contains 1 mg of product. Dilute in 75 µl of dry DMSO, giving you a 20mM solution. This solution is used as stock, it should be stored in –20℃ freezer

2. Add 2 µl of stock solution to 1 ml of culture medium to get a 40 µM final concentration ($1x10^6$cell count).

F. Z-Phe-Ala-FMK (Negative Control); M.W, 386;

1. This vial contains 1 mg of product. Dilute in 130 ul of dry DMSO, giving you a 20mM stock solution. Store in a -20℃ freezer

2. Use in place of Z-VAD-FMKor Z-DEVD-FMK as a negative control for inhibition of caspase activity.

Notes

- The release of free AFC from the peptide-AFC substrates can be measured either fluorometrically (ex: 400 nm; em: 505 nm) or spectrophotometrically (380 nm).
- Cell extracts for assay of caspase activites should contain approximately 1 X 10^6 cells/50 µl.
- Cell extracts can be prepared by 3 cycles of freeze thaw in assay buffer.
- The optimum concentration of peptide-FMK inhibitors is cell type dependent. Values reported in the literature to range from 5 uM to 100 µM.
- Concentrations of DMSO above 0.2% may be toxic to some cells.

STORAGE

Store at -20℃.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
910 Industry Drive, Seattle WA 98188
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com