Caspase-2 Fluorimetric Assay kit

KC-04-006

100/200/500 test

BQCkit

A brand of BioQuoChem

Index

All chemicals should be handled with care

- This kit is for R&D use only

Introduction

Caspases (cysteine-aspartic proteases, cysteine aspartases or cysteine-dependent aspartate-directed proteases) are a family of protease enzymes that play essential roles in programmed cell death. Caspase activity is measured by a short peptide containing specific cleavage sequences that are recognized by the caspase and are covalently attached to a colorimetric or fluorogenic detection probe. Initiator caspases (caspase 2, 8, 9 and 10) initiate the apoptosis signal and the executioner caspases (caspase 3, 6, and 7) carry out the mass proteolysis that leads to apoptosis.

Materials

BQCkit Caspase-2 Fluorimetric Assay kit *KC04006-100 test* contains:

Product	Quantity	Storage
Reagent A	1 vial	-20ºC
Reagent B	1 vial	-20ºC
Reagent C	1 vial	-20ªC

BQCkit Caspase-2 Fluorimetric Assay kit *KC04006-200 test* contains:

Product	Quantity	Storage
Reagent A	2 vials	-20ºC
Reagent B	2 vials	-20ºC
Reagent C	2 vials	-20°C

BQCkit Caspase-2 Fluorimetric Assay kit *KC04006-500 test* contains:

Product	Quantity	Storage
Reagent A	5 vials	-20ºC
Reagent B	5 vials	-20ºC
Reagent C	5 vials	-20°C

Assay Principle

BQC Caspase-2 Fluorometric Assay Kit provides a simple means for assaying the activity of caspases that recognize the sequence VDVAD. The fluorophore 7-amino-4-trifluoromethylcoumarin (AFC) after cleavage from the labeled substrate VDVAD-AFC is detected. The AFC can be monitored in a spectrofluorometer at an excitation wavelength of 400 nm and an emission wavelength range of 480-520 nm. Comparison of AFCf luorescence from an apoptotic sample vs. AFC fluorescence of control allows determination of the fold increase in caspase-2 activity.

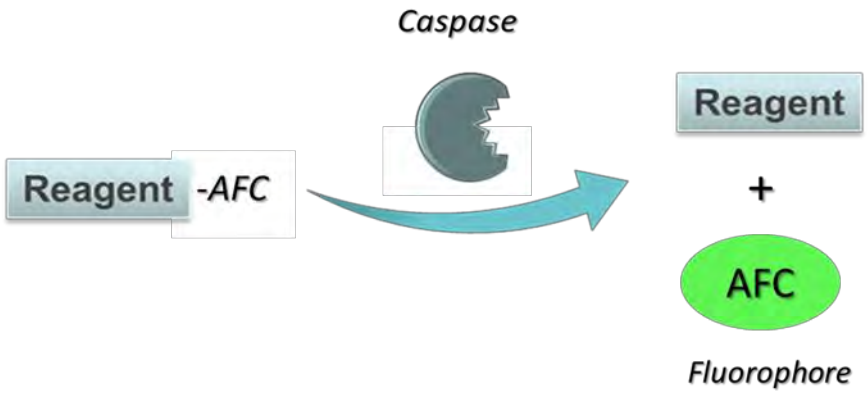


Figure 1. *Principle of the assay reaction*

Reagent Preparation

Reagent A (Cell Lysis Buffer):

Prepare 1x cell lysis buffer by diluting reagent A (10x cell lysis buffer) in ddH_2O. Example: Dilute 0.1 mL of reagent A in 0.9 mL of double distillated water and mix gently. Store at 4°C.

Reagent B (Reaction Buffer):

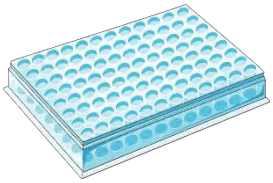
Prepare 1x reaction buffer by diluting reagent B (10x reaction buffer) in ddH2O. Example: Dilute 0.1 mL of reagent B in 0.9 mL of double distillated water and mix gently. Store at 4°C.

Reagent C (VDVAD-AFC):

Ready to use

Assay Protocol

Short protocol:

1 

Grow cells (adherent or suspension) and induce apoptosis in them by the desired method.

2 

Harvest cells, count and pellet them between 2 and $5x10^6$ cells in each sample.

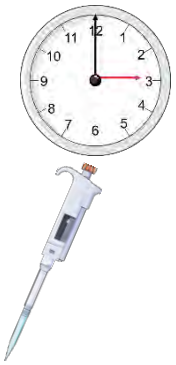
3 

Resuspend cells in 50 µL of previously diluted Reagent A (See Reagent Preparation). Incubate cells on ice for 10 minutes. Centrifuge for 1 min in a microcentrifuge (10,000xg) and transfer supernatant (cytosolic extract) to a fresh tube and put it on ice.

4 

Carry out a quantification of proteins in all samples (BQC Bradford Protein Quantification KB-03-003). Take the adequate volume to reach 100-200 µg of cytosolic extract and carry it to 50 µL with previously diluted Reagent A (See Reagent Preparation) for each assay.

5



Add 50 µL of previously diluted Reagent B (See Reagent Preparation) to each sample and 5 µL of Reagent C (VDVAD-AFC) substrate to each sample and incubate 1-2 hours at 37°C.

6

Read samples at an excitation wavelength of 400 nm and an emission wavelength range of 480-520 nm a spectrofluorometer using a 100 µL micro quartz cuvette or directly in a 96-well plate.

Data Analysis

Background reading from cell lysates and buffers should be subtracted from the readings. Caspase-2 activity can be determined by comparing the results of treated samples with the level of the uninduced control.

Warranties and Limitation of Liability

Bioquochem shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if Bioquochem has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, down time, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by Bioquochem´s gross negligence. Any and all liability of Bioquochem hereunder shall be limited to the amounts paid by buyer for product.

Buyer´s exclusive remedy and Bioquochem´s sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 6 months from the date of delivery

For further details, please refer to our website www.bqckit.com.