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CasPASE™ Colorimetric Apoptosis Assay

(Cat. # 786-858A to 786-863A; 786-858B to 786-863B)

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INTRODUCTION

The CasPASE™ Apoptosis Colorimetric Assay provides a simple and easy to follow method for assaying caspases 1-10, a key early indicator of apoptosis in mammalian cells. The assay is based on the detection of cleavage of a synthetic substrate, which is labeled with the chromophore ρ-nitroaniline (ρNA) at the C-terminal. When liberated from the peptide, ρNA produces an optical change that can be detected by reading the absorbance at 405nm. The reaction is selectively and irreversibly inhibited by the peptide Z-VAD-FMK. Comparison of the absorbance of an induced / apoptotic sample with an uninduced control allows one to determine the fold-increase in protease activity. The Caspase activity can also be quantitated by using a standard curve established with the free ρNA dye.

ITEM(S) SUPPLIED

Description	**50 Assay**	**100 Assay**
CasPASE™ Lysis Buffer [5X]	5ml	2 x 5ml
CasPASE™ Assay Buffer [5X]	10ml	2 x 10ml
ρNA Substrate Solution [2mM][ϒ]	0.5ml	2 x 0.5ml
Free Dye [10mM] (ρNA)	0.2ml	0.2ml
Caspase General Inhibitor (Z-VAD-FMK) [1mM]	0.1ml	2 x 0.1ml

[ϒ] The different substrate solutions supplied with individual kits are as follows:

Cat. #	**Assay** Substrate supplied	**Size**
786-858A 786-858B	**CasPASE™-1, 4, 5 Assay** Ac-WEHD-ρNA substrate	50 Assays 100 Assays
786-859A 786-859B	**CasPASE™-2 Assay** Ac-VDVAD-ρNA substrate	50 Assays / 100 Assays
786-860A 786-860B	**CasPASE™-3, 7,10 Assay** Ac-DEVD-ρNA substrate	50 Assays / 100 Assays
786-861A 786-861B	**CasPASE™-6 Assay** Ac-VEID-ρNA substrate	50 Assays / 100 Assays
786-862A 786-862B	**CasPASE™-8 Assay** Ac-IETD-ρNA substrate	50 Assays / 100 Assays
786-863A 786-863B	**CasPASE™-9 Assay** Ac-LEHD-ρNA substrate	50 Assays / 100 Assays

STORAGE CONDITION

The kit is shipped in blue ice. Store all reagents at -20°C. When used properly, these reagents are stable for 6 months. Buffers are stable for 1 year.

ADDITIONAL ITEMS NEEDED

- Centrifuge
- 96-well plates or Reaction Tube, etc.

PREPARATION BEFORE USE

Preparation of Kit Reagents

1. Allow the reagents to thaw into liquid form. Centrifuge the substrate, free dye and the inhibitor vials to collect the reagent solution at the bottom of the vial. Protect from light and humidity. Allow the reagents to reach RT before opening the vial.
2. Transfer an appropriate volume 5X CasPASE™ Lysis Buffer in a tube and dilute to 1X solution with pure water. i.e. Add 200µl 5X CasPASE™ Lysis Buffer to 800µl pure water.

Preparation of Cell Lysate

The following procedure is provided only as a suggestion.

1. Culture 10^7 cells under the appropriate conditions. Suspend cells in PBS or serum-free medium. For the attached cells, remove the cells from culture plate and suspend in PBS or serum-free medium. Pellet cells by centrifugation at 600xg for 5-6 minutes. Remove the supernatant cells and re-suspend the cells in PBS. If necessary, make cell counts. Re-pellet cells as before, remove and discard the supernatant. Lyse the cells by adding an appropriate volume of chilled Lysis Buffer e.g., 100µl Lysis each 1-5x 10^6 cells. Vortex gently to suspend cells.
2. Lyse the cells by freezing and thawing, 4-5 times. Do not vortex between freezes and thaws. Alternatively, after adding the Lysis Buffer, lyse the cells by passing the cell suspension 10-15 times through a 21gauge needle.

Preparation of Tissue Lysate

1. Homogenize 3-5mg tissue in 100µl Lysis Buffer.
2. Centrifuge the lysate for 30 minutes at full speed in a microfuge at 4°C. Collect the supernatant for the assay.

Preparation of CasPASE™ Assay Buffer

Immediately before use, transfer an appropriate volume of 5X CasPASE™ Assay Buffer in a tube and dilute to 1X solution with pure water. i.e. Add 200µl 5X CasPASE™ Assay Buffer to 800µl pure water.

Assay Controls

Prepare a negative control reaction with cells not treated with the apoptosis-inducing stimulus.

PROTOCOL

First read the section "Preparation before Use". The assay may be performed in a 96 well microplate or cuvette, using a colorimetric plate reader.

Set up the assay in duplicate and arrange the appropriate blanks and controls, such as a non-apoptotic cell lysate (negative control). A blank should be prepared to measure the substrate background and instrument drift.

1. Transfer the appropriate volume (see table) of 1X CasPASE™ Assay Buffer into each well.
2. Add 5µl of cell lysate into the appropriate wells as indicated in the table.
   NOTE: *For each assay, use lysate (5µl) obtained from at least 2x10^6 cells for colorimetric measurement. The use of fewer cells than this may reduce the observed increase of caspase activity.*
3. Add 10µl of 2mM ρNA Substrate Solution (0.2mM final concentration).
4. Mix the content of the wells and take a reading at zero time point (t = 0).
5. Cover the plate and incubate at 20-37°C
6. Measure the reaction by reading absorbance at 405nm every 30-60 minutes or until the measurements are significantly different from those at t=0.

Component	**Blank**	**Test Sample**	**Negative Control**
1X CasPASE™ Assay Buffer	90µl	85µl	85µl
Test Sample/Lysate	---	5µl	---
ρNA Substrate [2mM]	10µl	10µl	10µl
Negative Control/Lysate	---	---	5µl

Inhibition of Caspase Activity (Optional)

In order to establish non specific protease activity, a control should be run with or without the caspase specific inhibitor (Z-VAD-FMK) supplied with the kit.

1. Dilute the 1mM Inhibitor (Z-VAD-FMK) to 200µM before use in 1X CasPASE™ Assay Buffer. For example, add 10µl 1mM Inhibitor to 40µl 1X CasPASE™ Assay Buffer. The dilute inhibitor can be stored at -20°C.
2. Reaction tubes should be prepared as described above with 5µl Test Sample/ Lysate (see table below).
3. Add 10µl of the Inhibitor (10µM final concentration), mix and incubate the reaction at 20-37°C for 30 minutes to complete the inhibition.
4. Add 10µl of 2mM ρNA Substrate Solution (0.2mM final concentration).
5. Mix the content of the tube and take a reading at zero time point (t = 0).
6. Incubate the assay tubes at 20-37°C.
7. Measure the reaction by reading absorbance at 405nm every 30-60 minutes or until the measurements are significantly different from those at t=0.

Component	**Blank**	**Test Sample**
1X CasPASE™ Assay Buffer	90µl	75µl
Test Sample/Lysate	---	5µl
Inhibitor	---	10µl

CASPASE ACTIVITY CALCULATION

Colorimetric Assay

1. Dilute Free Dye [10mM] (ρNA) in CasPASE™ Assay Buffer to a stock concentration of 400µM by adding 4µl Free Dye [10mM] (ρNA) to 96µl CasPASE™ Assay Buffer
2. Prepare the standards as indicated in the table below:

Standard	Free Dye (ρNA)	CasPASE™ Assay Buffer (µl)	Final Conc.	
			(µM)	(nmoles)
A	20µl 400µM Stock from step 1	380	20	4
B	200µl Standard A	200	10	2
C	200µl Standard B	200	5	1
D	200µl Standard C	200	2.5	0.5
E	200µl Standard D	200	1.25	0.25
F	-	200	0	0

3. Generate the ρNA (ρ-Nitroaniline) dye calibration curve and determine the slope.
4. Plot nmole ρNA (x-axis) vs (Abs) Absorbance unit (y-axis) and determine slope i.e., OD/nmole ρNA.
5. Calculate the rate of increase in optical density (OD) for each sample as follows:

   $\Delta OD/\ minute = [\Delta OD_{sample} - \Delta OD_{blank}]$
   (i.e., change in OD over the length of the reaction time, minus the change in OD over the same length of reaction for the blank.)
6. Calculate the unit of caspase activity using the following formula:

   Units caspase in sample $= \Delta OD/\ minute \times (\text{calibration curve slope})^{-1}$

RELATED PRODUCTS

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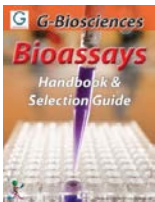

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