



G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

CasPASE™ Colorimetric Apoptosis Assay

(Caspase 2: Cat. # BAQ001, BAQ002, BAQ003)

(Caspase 3: Cat. # BAQ007, BAQ008, BAQ009)

(Caspase 6: Cat. # BAQ013, BAQ014, BAQ015)

(Caspase 9: Cat. # BAQ019, BAQ020, BAQ021)



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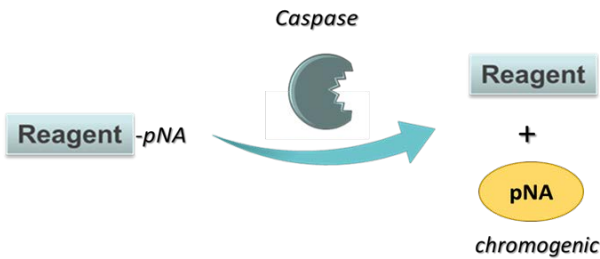
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INTRODUCTION

The CasPASE™ Apoptosis Colorimetric Assay provides a simple and easy to follow method for assaying caspases 2, 3,7, 10, , 6 and 9, 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 p-nitroaniline (pNA) at the C-terminal. When liberated from the peptide, pNA produces an optical change that can be detected by reading the absorbance at 405nm. Comparison of the absorbance of an induced / apoptotic sample with an uninduced control allows one to determine the fold-increase in protease activity.



ITEM(S) SUPPLIED

Description	100 Assay	200 Assay	500 Assays
CasPASE™ Lysis Buffer [10X]	1.5ml	2 x 1.5ml	5 x 1.5ml
CasPASE™ Assay Buffer [10X]	2ml	2 x 2ml	5 x 2ml
pNA Substrate Solution [2mM] ^Y	0.5ml	2 x 0.5ml	5 x 0.5ml

^Y The different substrate solutions supplied with individual kits are as follows:

Cat. #	Assay Substrate supplied	Size
BAQ001 BAQ002 BAQ003	CasPASE™ -2 Assay Ac-VDVAD-pNA substrate	100 Assays 200 Assays 500 Assays
BAQ007 BAQ008 BAQ009	CasPASE™ -3, 7,10 Assay Ac-DEVD-pNA substrate	100 Assays 200 Assays 500 Assays
BAQ013 BAQ014 BAQ015	CasPASE™ -6 Assay Ac-VEID-pNA substrate	100 Assays 200 Assays 500 Assays
BAQ019 BAQ020 BAQ021	CasPASE™ -9 Assay Ac-LEHD-pNA substrate	100 Assays 200 Assays 500 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 vial to collect the reagent solution at the bottom of the vial. Protect from light and humidity. Allow the reagents to reach ambient temperature before opening the vial.
2. Transfer an appropriate volume 10X CasPASE™ Lysis Buffer in a tube and dilute to 1X solution with pure water. i.e. Add 200µl 10X 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., 50µl Lysis each $1-5 \times 10^6$ 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 10X CasPASE™ Assay Buffer in a tube and dilute to 1X solution with pure water. i.e. Add 200µl 10X 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 and 1X CasPASE™ Lysis 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 2×10^6 cells for colorimetric measurement. The use of fewer cells than this may reduce the observed increase of caspase activity.
3. Add 5µl pNA Substrate Solution.
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	50µl	50µl	50µl
1X CasPASE™ Lysis Buffer	50µl	45µl	45µl
Test Sample/Lysate	---	5µl	---
pNA Substrate [2mM]	5µl	5µl	5µl
Negative Control/Lysate	---	---	5µl

CASPASE ACTIVITY CALCULATION

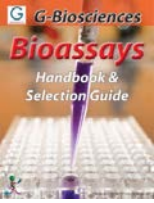
Colorimetric Assay

1. Calculate the rate of increase in optical density (OD) for each sample as follows:
$$\Delta OD / \text{minute} = [\Delta OD_{\text{sample}} - \Delta OD_{\text{blank}}]$$

(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.)
2. Compare the $\Delta OD / \text{minute}$ of caspases in the induced and uninduced (negative control) samples.

RELATED PRODUCTS

Download our Bioassay Handbook



<http://info.gbiosciences.com/complete-bioassay-handbook>

For other related products, visit our website at www.GBiosciences.com or contact us.

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