



Apr 01, 2025

## Caspase 3/7 Activity

DOI

<https://dx.doi.org/10.17504/protocols.io.x54v97mkzg3e/v1>

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**Protocol Citation:** Karyna Tarasova, Sinan Gültekin, iris.gerner Gerner, Florian Jenner 2025. Caspase 3/7 Activity. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.x54v97mkzg3e/v1>

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**Protocol status:** Working

We use this protocol and it's working

**Created:** April 01, 2025



**Last Modified:** April 01, 2025

**Protocol Integer ID:** 125870

**Keywords:** Senescence, Caspase 3/7, Apoptosis, cell based assay, caspase activity, caspase, luminescent assay, cell lysis, tetrapeptide sequence devd, assay, luminescence, cell

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## Abstract

The Caspase-Glo 3/7 Assay is a homogeneous, luminescent assay that measures caspase-3 and -7 activities. The assay provides a luminogenic caspase-3/7 substrate, which contains the tetrapeptide sequence DEVD, in a reagent optimized for caspase activity, luciferase activity and cell lysis. Luminescence is proportional to the amount of caspase activity present.

## Materials

- multichannel pipette or automated pipetting station
- plate shaker for mixing multiwell plates
- luminometer capable of reading multiwell plates



## Before start

The Caspase Glo 3/7 Assay was acquired from Promega (Madison, USA).

1. Equilibrate the Caspase-Glo® 3/7 Buffer and lyophilized Caspase-Glo® 3/7 Substrate to room temperature before use.

2. Transfer the contents of the Caspase-Glo® 3/7 Buffer bottle into the amber bottle containing Caspase-Glo® 3/7

Substrate. Mix by swirling or inverting the contents until the substrate is thoroughly dissolved to form the Caspase-Glo® 3/7 Reagent.

Storage: The reconstituted Caspase-Glo® 3/7 Reagent may be stored at 4°C for up to 3 days with no loss of activity compared to that of freshly prepared reagent. Reconstituted reagent stored at 4°C for 1 week will give a signal approximately 90% of that obtained with freshly prepared reagent, while reconstituted reagent stored at 4°C for 4 weeks will give a signal approximately 75% of that obtained with freshly prepared reagent.

Reconstituted reagent that has been refrozen and stored at –20°C for 1 week will give a signal approximately 75% of that of freshly prepared reagent, and refrozen reagent stored at –20°C for 4 weeks will give a signal approximately 60% of that of freshly prepared reagent.



## Reagent Preparation

1

- 1.1 Equilibrate the Caspase-Glo 3/7 Buffer and lyophilized Caspase-Glo 3/7 Substrate to room temperature before use. 
- 1.2 Transfer the contents of the Caspase-Glo 3/7 Buffer bottle into the amber bottle containing Caspase-Glo 3/7 Substrate. Mix by swirling or inverting the contents until the substrate is thoroughly dissolved to form the Caspase-Glo 3/7 Reagent. 
- 1.3

## Detection of Caspase-3 and -7 Activities

2

- 2.1 Prepare and mix all reagents thoroughly before use according to instructions. Each unknown sample should be assayed in duplicate or triplicate. 
- 2.2 Remove the media from all wells and discard.
- 2.3 Mix 1:1 ratio Caspase-Glo 3/7 Reagent volume to medium and add 100μL of the mix in each well. Incubate for 3h at 37°C in the incubator.  
- 2.4 Measure the luminescence of each sample in a plate-reading luminometer as directed by the luminometer manufacturer.  
- 2.5 Normalize Caspase 3/7 enzymatic activity to total protein concentrations.

## Total protein concentration

3



- 3.1 Total protein concentrations was measured by Qubit Protein Assay Kit (ThermoFisher, Massachusetts USA) using the Qubit 4 Fluorometer (Thermo Fisher Scientific, Massachusetts USA).

