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, Florien Jenner 2025. Caspase 3/7 Activity. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v97mkzg3e/v1

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 lysi, tetrapeptide sequence devd, assay, luminescence, cell

Disclaimer

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^{o}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).