Caspase 3/7 Assay Kit

Catalog No.: abx294003

Size: 100 tests

Detection Range: 12.5 µmol/L – 200 µmol/L

Sensitivity: 12.5 µmol/L

Storage: Store all components at -20°C. Store the Substrate and Standard in the dark.

Application: For detection and quantification of Caspase 3/7 activity in tissue homogenates and cell lysates.

Introduction

Abbexa's Caspase 3/7 Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Caspase 3/7 activity. Caspase 3/7 in samples catalyzes the Substrate to produce yellow-colored p-Nitroaniline. The yellow product has an absorbance maximum at 405 nm. The intensity of the color is proportional to the Caspase 3/7 activity, which can then be calculated.

Kit components

1. Lysis Buffer: 50 ml
2. Reaction Buffer (2X): 50 ml
3. Substrate: 0.5 ml
4. Standard (10 mmol/L): 1 ml

Materials required but not provided

1. Spectrophotometer (405 nm)
2. PBS (0.01 M, pH 7.4)
3. Normal saline (0.9 % NaCl)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. Vortex mixer
8. Incubator

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Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh 50 mg of tissue and wash with PBS (0.01 M, pH 7.4) or normal saline (0.9 % NaCl). Cut into small pieces and add 200 µl of pre-cooled Lysis Buffer, then homogenize the sample on ice. Transfer the homogenate to a fresh tube and stand on ice for 30 minutes. Centrifuge at 11,000 × g for 10-15 minutes at 4°C, collect the supernatant and transfer to a fresh tube and store on ice for immediate detection. The protein content of the supernatant should be determined separately using the Bradford method **(abx097194)**.
- **Cell Lysates:** Harvest 1 × 10^6 cells and resuspend in PBS (0.01 M, pH 7.4). Centrifuge at 600 × g for 5 minutes and discard the supernatant. Resuspend the cell pellet in 100 µl of pre-cooled Lysis Buffer per 10^6 cells, then stand on ice for 30 minutes, mixing for 10 seconds for a total of 3-4 times during the 30 minutes. Centrifuge at 11,000 × g for 10-15 minutes at 4°C, collect the supernatant and transfer to a fresh tube and store on ice for immediate detection. The protein content of the supernatant should be determined separately using the Bradford method **(abx097194)**.

  Note: If testing adherent cell cultures, any suspension cells produced during preparation should also be collected for lysis.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- The recommended protein content for assay is 1 mg/ml – 4 mg/ml

2. Reagents

- **Standard Diluent:** Mix Lysis Buffer and Reaction Buffer (2X) to a 1:1 ratio, mix thoroughly.
- **Standards:** Label 6 tubes with 200 µmol/L, 100 µmol/L, 50 µmol/L, 25 µmol/L, 12.5 µmol/L, and 0 µmol/L (Blank). Add 294 µl of prepared Standard Diluent to the 200 µmol/L tube and 150 µl of prepared Standard Diluent all other tubes. Add 6 µl of 10 mmol/L Standard to the 200 µmol/L tube and mix thoroughly. Transfer 150 µl of solution from the 200 µmol/L tube to the 100 µmol/L tube and mix thoroughly. Repeat the transfer of 150 µl of solution between tubes to perform the serial dilution, stopping at the 12.5 µmol/L tube. The 0 µmol/L tube should only contain the initial 150 µl of prepared Standard Diluent.

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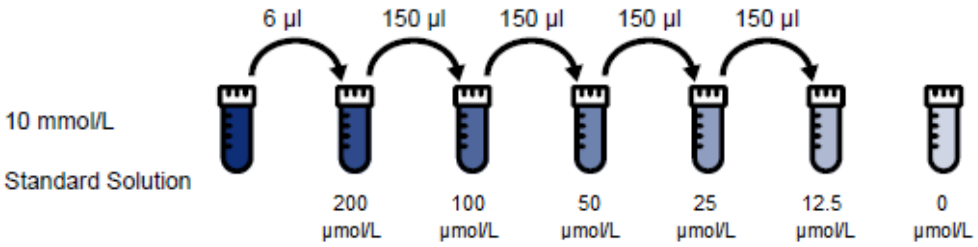

Note:

- Store all reagents on ice during use.

B. Assay Procedure

Standard Curve:

1. Add 100 µl of standard dilutions (including blank) to sterile microcentrifuge tubes.
2. Measure the OD of each tube using optical path cuvettes with a spectrophotometer at 405 nm.

Samples:

1. Mark microcentrifuge tubes for each sample and blank. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 45 µl of 2X Reaction Buffer to sample and blank tubes.
3. Add 50 µl of sample to sample tubes, add 50 µl of Lysis Buffer to blank tubes
4. Mix gently, then add 5 µl of Substrate to each tube and mix thoroughly. Avoid foaming.
5. Incubate at 37°C for 1-2 hours, if no color change is observed after 2 hours, incubation can be extended up to 4 hours if necessary.
6. Measure the OD of each tube using optical path cuvettes with a spectrophotometer at 405 nm.

C. Calculation of Results

Plot the standard curve, using the mean OD of duplicate standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the activity of Caspase 3/7 in each sample well can be derived using 2 methodologies, using the following formulae:

1. Calculated enzyme activity:

One unit of Caspase 3/7 activity is defined as the amount required to catalyze 1 nmol of Substrate per hour at 37°C under saturated substrate conditions.

$$\text{Caspase 3/7 activity (U/mg protein)} = \frac{\Delta A_{405} - b}{a} \times \frac{V_1}{V_2 \times C_{\text{Protein-Sample}} \times T} \times f$$

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2. **Relative increase in enzyme activity:**

If a negative biological control sample is tested, the percentage increase in activity can be calculated as follows:

$$\text{Caspase 3/7 activity (100\%)} = \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Negative} - OD_{Blank}}} \times \frac{\mathbf{C_{Protein-Negative}}}{\mathbf{C_{Protein-Sample}}} \times \mathbf{100\%}$$

where:

ΔA_{405}	$OD_{Sample} - OD_{Blank}$
$OD_{Negative}$	OD value of negative control (Method 1)
OD_{Blank}	OD value of blank sample
V_1	Total volume of the reaction (0.1 ml)
V_2	Sample volume of the reaction (0.05 ml)
$C_{Protein-Sample}$	Concentration of protein in sample (mg protein/ml)
$C_{Protein-Negative}$	Concentration of protein in negative control sample (mg protein/ml)
T	Reaction time (hours)
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
f	Sample dilution factor

D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.

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