

## Caspase detection protocol

### Introduction

Caspases are essential in cells for apoptosis, one of the main types of programmed cell death in development and most other stages of adult life, and have been termed "executioner" proteins for their roles in the cell. Caspases were first implicated in apoptosis when CED-3, a protein required for programmed cell death in *Caenorhabditis elegans*, was found to have close homology with the mammalian interleukin-1 $\beta$ -converting enzyme (ICE or caspase 1) and that over-expression of ICE induced apoptosis. Failure of apoptosis is one of the main causes of tumour development and autoimmune diseases. This coupled with the unwanted apoptosis that occurs with ischaemia or Alzheimer's disease, has raised interest in caspases as potential therapeutic targets.

Caspases are enzymes known as proteases, which play essential roles in apoptosis and inflammation. As proteases, they are enzymes that cleave other proteins. They are called cysteine proteases, because they use a cysteine residue to cut those proteins, and called caspases because the cysteine residue cleaves their substrate proteins at the aspartic acid residue. The generic name for all members is caspase with the *c* denoting a cysteine protease and *aspase* referring to the aspartate specific cleaving ability of these enzymes. The individual members are then numbered according to their chronological order of publication. To date 14 caspases have been identified (12 human and 2 murine).

### Types of caspase proteins

**Initiator caspases** (e.g. *CASP1*, *CASP2*, *CASP8*, *CASP9*, *CASP10*, *CASP12*) cleave inactive pro-forms of effector caspases, thereby activating them.

**Effector caspases** (e.g. *CASP3*, *CASP6*, *CASP7*, *CASP14*) in turn cleave other protein substrates within the cell resulting in the apoptotic process. The initiation of this cascade reaction is regulated by caspase inhibitors.

### Immunohistochemical staining protocol

Materials required:

- Active Caspase 3 antibody, rabbit pAb (ab2302)
  - prepared, fixed samples on slides
  - Triton X-100
  - PBS
  - blocking buffer (PBS/0.1% Tween 20 + 5% horse serum)
  - goat anti-rabbit Cy5 conjugate secondary antibody (ab6564)
  - mounting medium
  - humidified chamber
1. Permeabilize the fixed samples by incubating in PBS/0.1% Triton X-100 for 5 mins at room temperature.
  2. Wash three times in PBS, for 5 mins at room temperature.

3. Drain the slide and add 200 µl of blocking buffer (PBS/0.1% Tween 20 + 5% horse serum). Use of serum from the host species of the conjugate antibody (or closely related species) is suggested. Lay the slides flat in a humidified chamber and incubate for 1-2 hours at room temperature. Rinse once in PBS.
4. Add 100 µl of the active Caspase 3 antibody diluted 1/200 in blocking buffer. You can also prepare a slide with no active Caspase 3 as a negative control. Incubate slides in a humidified chamber overnight at 4 °C.
5. The following day, wash the slides three times, 10 mins each in PBS/0.1% Tween 20 at room temperature.
6. Drain slides and add 100 µl of goat anti-rabbit Cy5 conjugate diluted 1:500 in PBS. Lay the slides flat in a humidified chamber, protected from light, and incubate for 1-2 hours at room temperature. Wash three times in PBS/0.1% Tween 20 for 5 mins, protected from light.
7. Drain the liquid, mount the slides in a permanent or aqueous mounting medium and observe with a fluorescence microscope.

Abcam have many caspase antibodies and kits available. The above is a suggested protocol only which may require optimization. Customers will need to use the protocols provided when using Caspase kits:

**Caspase detection kits:**

[ab39470 Caspase 1 Colorimetric Assay Kit](#)  
[ab39412 Caspase 1 Fluorometric Assay Kit](#)  
[ab39830 Caspase 2 Colorimetric Assay Kit](#)  
[ab39794 Caspase 2 Fluorometric Assay Kit](#)  
[ab39401 Caspase 3 Colorimetric Assay Kit](#)  
[ab39383 Caspase 3 Fluorometric Assay Kit](#)  
[ab39709 Caspase 6 Colorimetric Assay Kit](#)  
[ab39707 Caspase 6 Fluorometric Assay Kit](#)  
[ab39700 Caspase 8 Colorimetric Assay Kit](#)