

## Caspase 3 Activity Assay

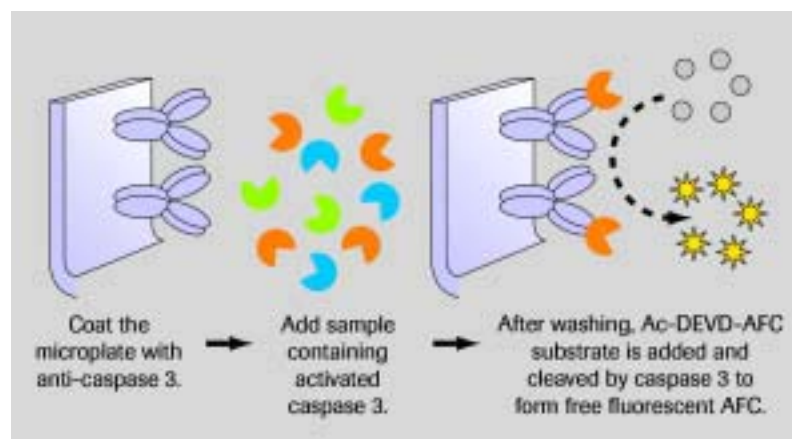
Cat. No. 12 012 952 001 96 tests

<b>Type</b>	Immunosorbent enzyme assay, fluorometric
<b>Useful for</b>	Specific, quantitative <i>in vitro</i> determination of caspase 3 activity
<b>Samples</b>	Cell lysates, recombinant caspase 3 (CPP32)
<b>Method</b>	Cell lysis, followed by capturing of caspase 3 by a specific antibody and fluorometric determination of proteolytic cleavage of the substrate
<b>Time</b>	Approx. 5 h (after induction of apoptosis)

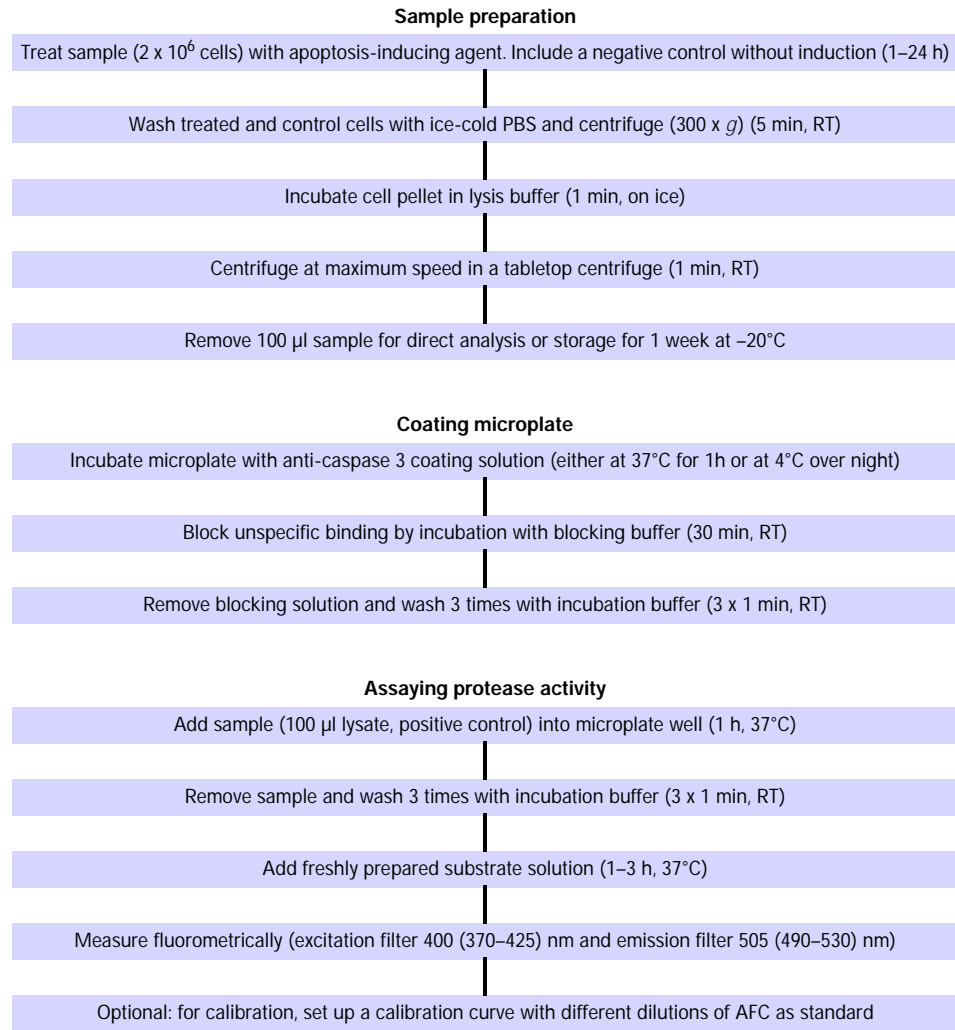
**Significance of kit:** This kit allows specific, quantitative detection of caspase 3 activity in cellular lysates after induction of apoptosis. Caspase 3 activation play a key role in initiation of cellular events during the early apoptotic process. The immunosorbent enzyme assay principle of this kit guarantees high specificity without cross-reactions with other known caspases. The fluorochrome generated by proteolytic cleavage of the caspase substrate is proportional to the concentration of activated caspase 3 in the lysates.

**Test principle:** The assay uses a fluorometric immunosorbent enzyme assay (FIENA) principle. The procedure (Figure 13 and Flow Chart 4) involves:

- 1 Inducing apoptosis in cells by desired method (for instance  $2 \times 10^6$  cells). After the induction, the cells are washed and pelleted by centrifugation.
- 2 Preparing samples by resuspending and incubating cells in lysis buffer. After lysis and following centrifugation, samples can be removed for direct analysis or storage.
- 3 Coating microplate with anti-caspase 3 solution and blocking of unspecific binding.
- 4 Transferring a sample to the anti-caspase 3-coated well of a microplate and capturing of caspase 3.
- 5 Washing the immobilized antibody-caspase 3 complexes three times to remove cell components that are not immunoreactive.
- 6 Incubating sample with caspase substrate (Ac-DEVD-AFC) that is proteolytically cleaved into free fluorescent AFC.
- 7 Measuring generated AFC fluorometrically.



▲ Figure 13: How the Caspase 3 Activity Assay works.



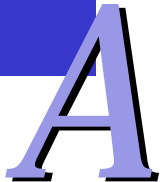
▲ **Flow Chart 4: Assay procedure, Caspase 3 Activity Assay.**

**Sensitivity:** In a model system, caspase 3 activity was clearly detectable in lysates of 10<sup>6</sup> cells with 5 % apoptotic cells (Figure 14). However, the lower limit for determination of caspase 3 activity in cellular lysates of dying cells in a particular sample varies with the kinetics of the apoptotic process, the apoptotic agent used, and the number of affected cells within the total cell population.

**Specificity:** This fluorometric immunosorbent enzyme assay is highly specific for caspase 3 by the use of an anti-caspase 3-specific monoclonal capture antibody in combination with a specific caspase substrate. Enzyme activity of natural and recombinant human caspase 3 is detected by this assay. Cross-reactions with other caspases are not known.

**Can be used to assay:**

- Lysates of adherent cells, of cells in suspension culture, of cells obtained *ex vivo* or recombinant caspase 3.



**Kit contents**

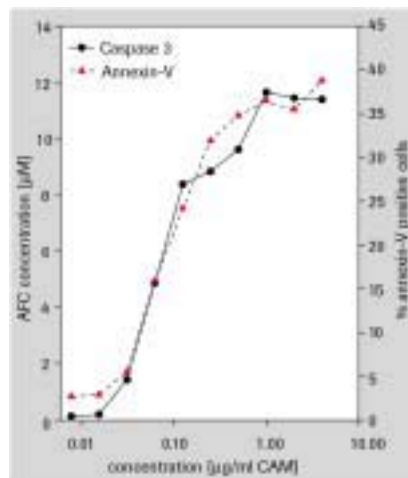
1. Coating buffer, 10x
2. Anti-caspase-3, 20x
3. Blocking buffer, ready-to-use
4. Incubation buffer, 5x
5. DTT, 100x
6. Substrate solution Ac-DEVD-AFC, 20x
7. AFC
8. Positive control, apoptotic U937 cell lysate
9. Microplate modules (12 x 8-wells)
10. Adhesive plate cover

**Typical results:** see Figures 14–15

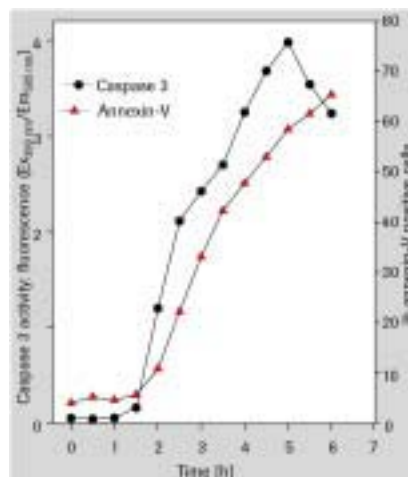
**Other applications:** For more examples of how the Caspase 3 Activity Assay can be used in the lab, see Appendix, pages 132–133.

The caspase 3 activity assay has been used to detect caspase 3 activation in U937 cells exposed to different concentrations of the apoptosis inducing agent camptothecin (CAM) (Figure 14, dose response curve). In this model system, the induction of apoptosis in only 5% of U937 cells is sufficient for detection of caspase 3 activation. Caspase 3 activity/fluorochrome development is proportional to the percentage of apoptotic cells.

Figures 14 and 15 demonstrate that Caspase 3 activity and Annexin-V binding correlate very closely in both dose-response and kinetic studies.



◀ **Figure 14: Dose-response experiment analyzed by the caspase 3 Activity Assay.** U937 cells were exposed to different concentrations of camptothecin (CAM) for 4 h at 37°C. Lysates were analyzed for caspase 3 activity and standardized values are plotted versus concentration. Additionally, an aliquot of the same cells was analyzed for Annexin-V binding.



◀ **Figure 15: Kinetic study of caspase 3 activation by camptothecin exposure in U937 cells.** U937 cells were exposed to 4  $\mu\text{g/ml}$  camptothecin for different time intervals at 37°C. Lysates were analyzed for caspase 3 activity and fluorescence (minus fluorescence of blank) is plotted versus time. Additionally, an aliquot of the same cells was analyzed for Annexin-V binding in parallel.