

## Homogeneous Caspases Assay

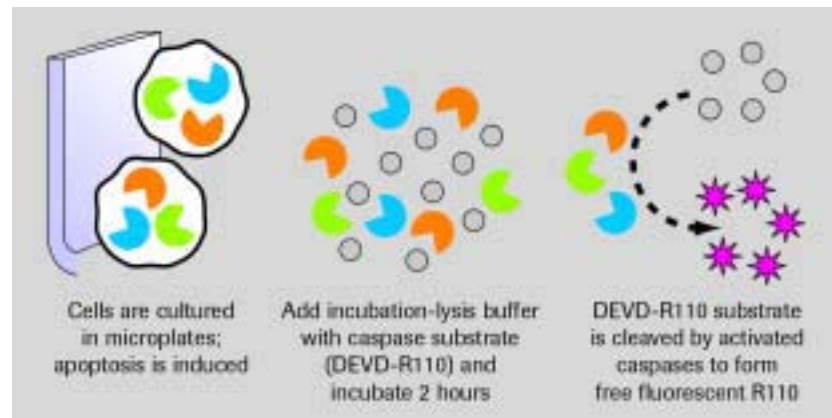
Cat. No. 03 005 372 001 100 assays (96-well plates)  
400 assays (384-well plates)

Cat. No. 12 236 869 001 1000 assays (96-well plates)  
4000 assays (384-well plates)

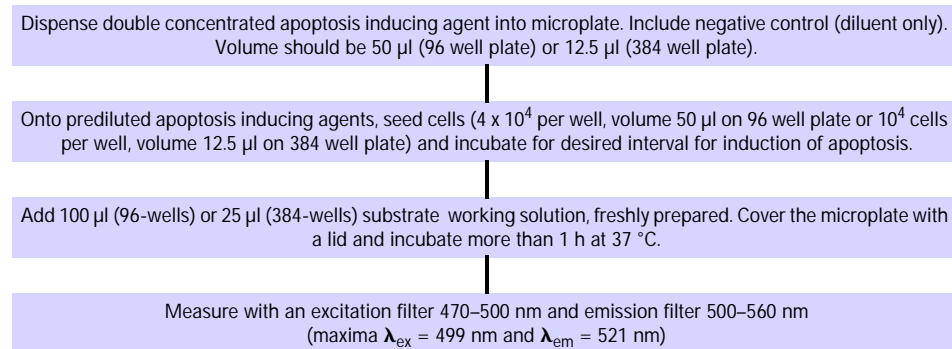
<b>Type</b>	One step assay, fluorimetric
<b>Useful for</b>	Specific, quantitative <i>in vitro</i> determination of caspases in microplates
<b>Samples</b>	Cell cultures, recombinant caspases
<b>Method</b>	Cell lysis, followed by detection of caspases activity (fluorimetric determination of proteolytic cleavage of the substrate)
<b>Time</b>	Approx. 2 h (after induction of apoptosis)

**Significance of kit:** The Homogeneous Caspases Assay is a fluorimetric assay for the quantitative *in vitro* determination of caspases activity in microplates, which makes it especially useful for high throughput screening. Apoptotic cells are incubated with DEVD-Rhodamine 110 for 1–24 h. Upon cleavage of the substrate by activated caspases, fluorescence of the released Rhodamine 110 is measured.

**Test principle:** The kit can be used for the quantification of activated caspases of human as well as animal origin, or screening for caspase inhibitors. It is one step assay, including the cell lysis step.



▲ Figure 16: How the Homogeneous Caspases Assay work.



▲ Flow Chart 5: Assay procedure, Homogeneous Caspase Assay.

**Specificity:** Specifically detects caspases 2, 3 and 7, caspases 6, 8, 9 and 10 to a lesser extent

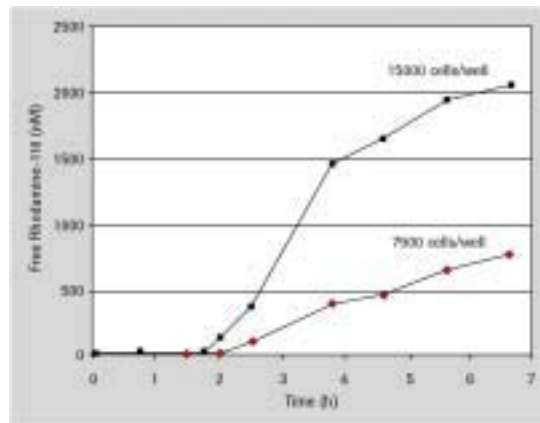
**Can be used to assay:** Cell cultures, recombinant caspases

**Kit contents**

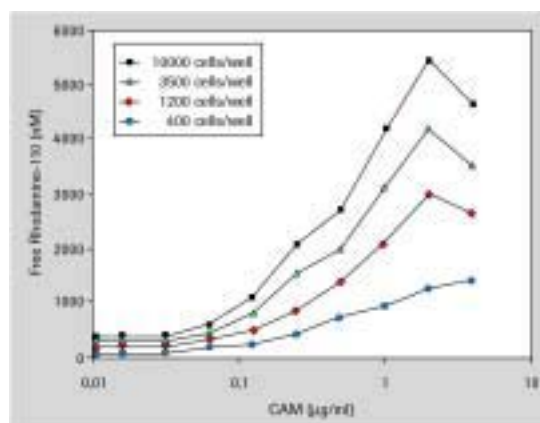
1. Substrate stock solution, 10x
2. Positive control, 10x
3. Rhodamine 110, standard
4. Incubation buffer

**Typical results:** see Figures 17–18

**Other applications:** For more examples of how the Homogeneous Caspases Assay can be used in the lab, see Appendix, page 133.



**Figure 17:** Kinetics of caspase activation in U937 cells by camptothecin (384-well plate). U937 cells were exposed to 4 µg/ml camptothecin for different time intervals at 37 °C, analyzed for caspase activity with the Homogeneous Caspases Assay and fluorescence plotted versus time.



**Figure 18:** Dose-response curve of U937 cells exposed to different concentrations of camptothecin (CAM) (384-well plate). U937 cells were exposed to camptothecin for 4 h at 37 °C, analyzed for caspase activity with the Homogeneous Caspases Assay and standardized values plotted versus concentration.