

# M30-Apoptosense™ ELISA

## For research use only

A new tool for quantification of apoptosis in cultured cells

A simple and reliable method, the M30-Apoptosense™ ELISA, is now available to quantify apoptosis in cell extracts and tissue culture supernatants.

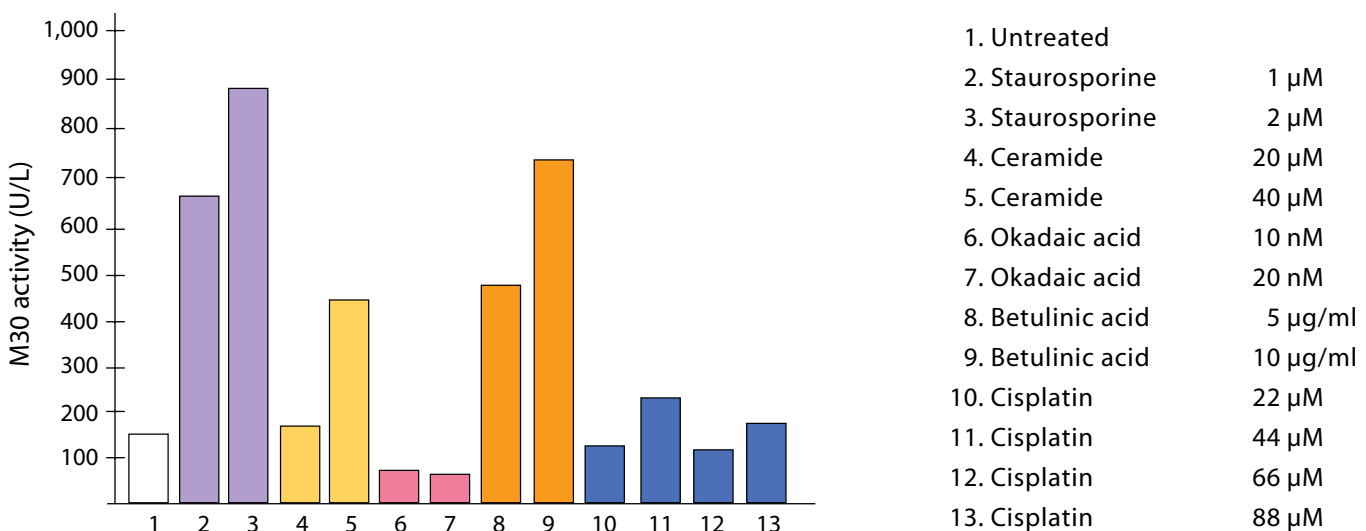
- + The M30-Apoptosense™ ELISA is specific for apoptosis
- + The M30-Apoptosense™ ELISA is sensitive, robust and easy to perform (detects a signal from less than 5 000 epithelial cells)
- + The M30-Apoptosense™ ELISA is in a 96-well microtiter format assisting in fast drug candidate screening and kinetic studies

### I. Drug screening of pro-apoptotic agents

The sensitivity of a breast cancer cell line to different agents was determined in vitro. The agents were added to cell cultures and after 18 hours of incubation, the supernatants were measured using the M30-Apoptosense™ ELISA kit. At this time point (18 hours) the agents showed very remarkable differences with regard to their ability to induce apoptosis.

Particularly striking was the low induction by cisplatin at different concentrations within the first hours. However, as shown in the next section (figure 2), cisplatin induces apoptosis after further incubation (36 - 48 hours). Also, there seems to be no M30 antigen activity within 18 hours using okadaic acid.

Figure I: Induction of apoptosis with pro-apoptotic agents



MCF-7 breast cancer cells were seeded with 100,000 cells per plate in 1.5 ml medium and incubated with various agents for 18 hours. Samples (25μL) were collected from the supernatant and M30 antigen was measured using the M30-Apoptosense™ ELISA.

## 2. Time course kinetics of apoptosis

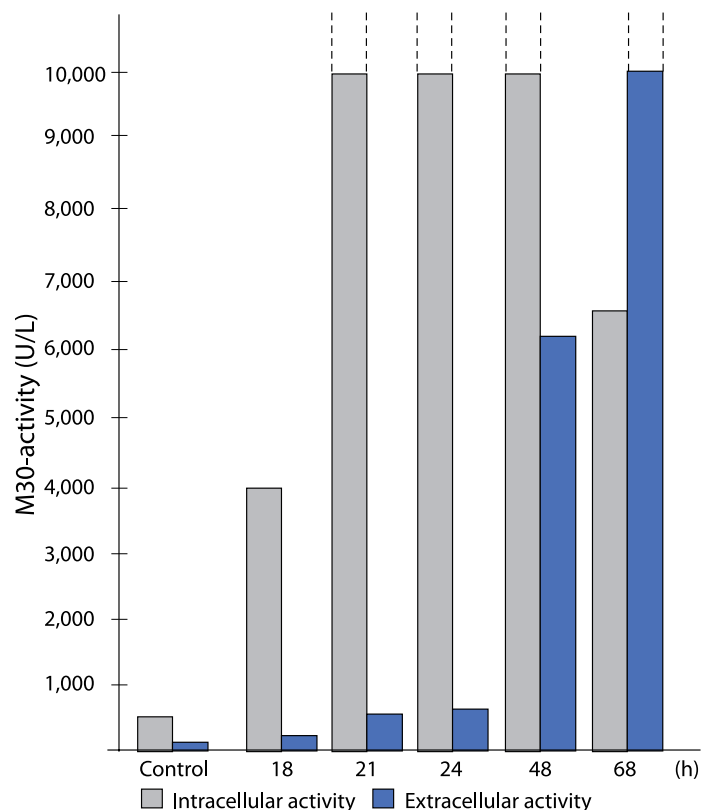
The time course kinetics of apoptosis induction was studied using intracellular (cell extracts) and extracellular (culture supernatants) samples from cultures of a breast cancer cell line. Cell extracts and culture supernatants were collected at different time points and measured with the M30-Apoptosense™ ELISA.

An increase in intracellular M30-activity is observed prior to increases in the culture supernatant. The increase in the culture medium parallels the loss of membrane integrity.

For drug candidate screening, it is convenient to add non-ionic detergents to the cell cultures to assay the combined activity in extracts and medium.

MCF-7 breast cancer cells (Figure 2) were seeded at 100,000 cells per 1.5 ml medium and incubated with cisplatin (50µM). Samples (25µl) were collected from the supernatant and M30 antigen was measured using the M30-Apoptosense™ ELISA. Extracts were prepared by lysing cells (in 10 mM Tris-HCl, pH 7.4 / 10 mM MgCl<sub>2</sub> / 150 mM NaCl / 0.5% NP-40) and M30-activity was measured.

Figure 2: Cisplatin induced apoptosis in MCF-7 cells



## 3. Caspases and M30-activity

The relationship between caspases and M30-activity was shown by the following experiment: The M30-activity was measured in a paralleled experiment with and without the caspase-inhibitor z- VAD-fmk. This result confirms that the increase of the M30 neo-epitope is mediated by caspases. Therefore, caspase activity can be determined also by measurement of the M30-activity.

MDA-MD-231 cells were treated with cisplatin (40µM) for 48 hours in the presence or absence of z- VAD-fmk (50µM). Activity was assayed in tissue culture supernatants (Figure 3).

Figure 3: The increase in M30-activity induced by cisplatin is blocked by z- VAD-fmk

