

M30-Apoptosense™ ELISA

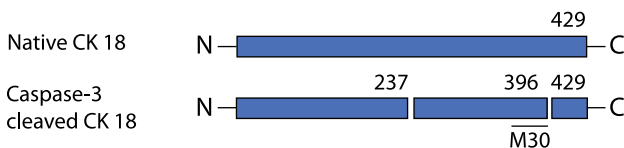
For research use only

A sensitive and apoptosis-specific assay

Apoptosis is a biochemically and morphologically distinct form of cell death. One of the key steps in apoptosis is triggered by the action of proteases, called caspases.

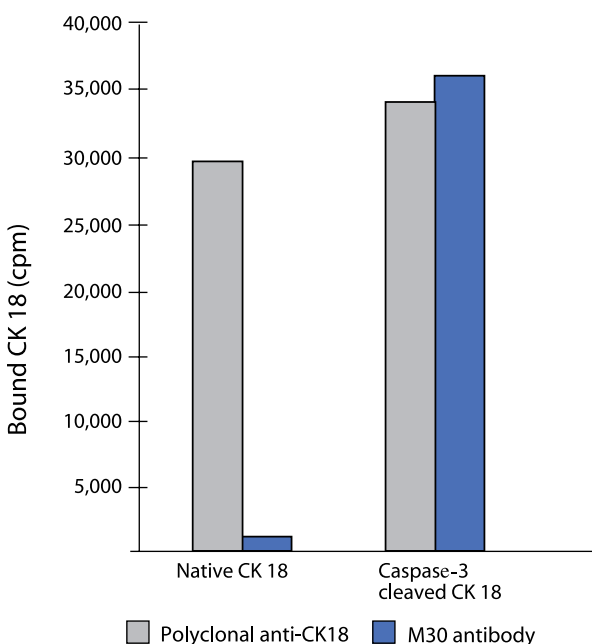
The M30 monoclonal antibody specifically recognizes apoptotic, not necrotic or viable, epithelial cells. This is due to its recognition of a neo-epitope in the C-terminal domain of cytokeratin 18 (CK 18) which is exposed after cleavage by caspases during apoptosis (Leers et al, J Pathology 187, 567-572, 1999) (Figure 1). M30-reactivity is observed prior to TUNEL or Annexin V reactivity (Leers et al, 1999).

Figure 1: Cleavage of CK 18 by caspases during apoptosis



The specificity of M30 towards caspase cleaved CK 18 is illustrated in figure 2. In this experiment, native and caspase-3 cleaved CK 18 were incubated with antibody coated beads. Whereas a polyclonal antibody binds both proteins, the M30 MAb showed only reactivity with respect to the caspase-3 cleaved CK 18.

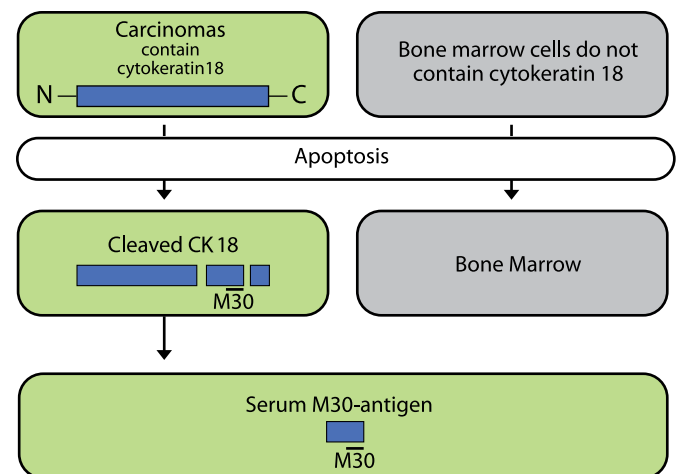
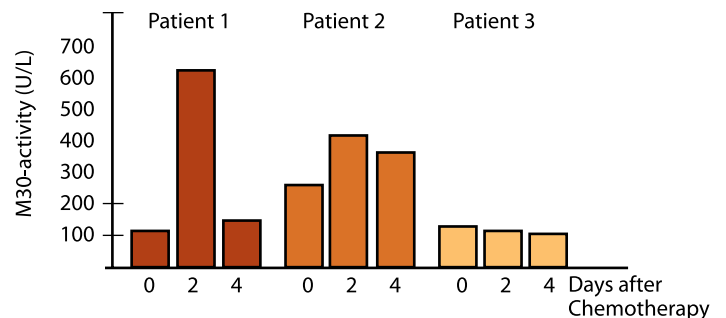
Figure 2: M30 MAb specifically binds caspase cleaved CK 18



The M30-Apoptosense™ ELISA, based on the M30 antibody, has been developed. In this assay, a monoclonal antibody is used as a catcher for all CK 18 molecules, its fragments and especially the neo-epitope recognized by the M30 MAb. The neo-epitope is then specifically determined by the horseradish peroxidase-labelled M30 antibody.

A powerful potential research application of the M30-Apoptosense™ ELISA is measuring apoptosis induction after exposure of cancer cells to chemotherapy/ radiation with respect to the base value before exposure. In some sera, increases in M30-activity are observed after exposure. (Linder et al., Tumor Biology 21 (suppl. 1) p. 30, 2000).

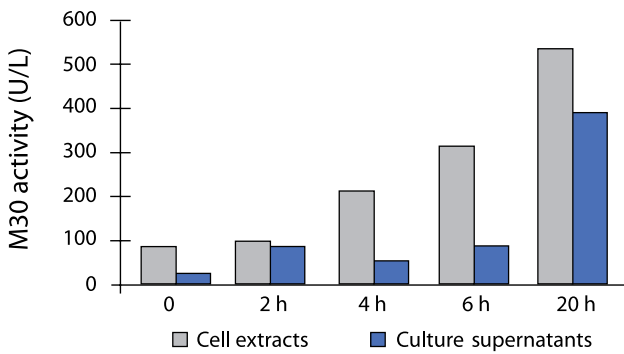
Figure 3: M30-activity in sera of cancer cells exposed to chemotherapy agents



Successful chemotherapy will induce apoptosis of both tumor cells and white blood cells. Carcinomas of epithelial origin like breast, lung, colon, prostate express cytokeratin 18, whereas white blood cells do not. M30 antigen levels will therefore reflect apoptosis of carcinoma cells.

The M30-Apoptosense™ ELISA can be used in research to monitor apoptosis in vitro (Figure 4). This was shown by treating a human breast cancer cell line with the pro-apoptotic agent staurosporine. Cell extracts and culture supernatant samples were collected at different time points. An increase in **intracellular** activity is observed after 4 hours. Increased **extracellular** activity is observed later, when membrane integrity has been lost.

Figure 4: Increases in M30-activity in cell extracts and culture supernatants during staurosporine-induced apoptosis



Research has shown similar types of results with different pro-apoptotic agents (genotoxins, cytotoxic agents, inhibitors) in different cell lines making the M30-Apoptosense™ ELISA a very useful tool in studies and follow-up of apoptosis.

A particularly useful application is screening for pro-apoptotic drugs that may be used in future cancer therapy.

The M30 antibody is a specific mouse monoclonal IgG2b antibody against the CK 18 neo-epitope and reacts both with human, monkey, and bovine apoptotic cells.

The M30-epitope has been defined (Leers et al., 1999) using synthetic peptides. The strongest binding activity is observed towards peptides with the caspase cleavage site DALD at the C-terminus. Addition or deletion of one amino acid reduces binding.

Figure 5: Mapping of the M30 epitope

Peptide	383	396	Bindings(%)
LEDGEDFNLGDALDS			100
L-----D			5
L-----L			13

The increase of M30-activity in vitro has been shown to be dependent on caspase activity. Figure 6 shows that the increase of M30-activity observed after exposure of breast cancer cells to cisplatin is blocked subsequent to treatment with the caspase inhibitor z-VAD-fmk.

Figure 6: Increases in M30-activity during apoptosis are due to caspase activity

