

Apoptosis Measurement – Method Comparison

	M30-APOPTOSENSE	TUNEL	ANNEXIN-V	PARP	CASPASE ACTIVITY
Measurement Principle	Novel ELISA kit is based on the ability of the M30 monoclonal antibody to recognize a neo-epitope on cytokeratin 18 that becomes exposed after cleavage by caspases during apoptosis. Formatted in a reagent complete, convenient 96-well ELISA kit.	TUNEL (terminal deoxynucleotidyl transferase nick-end labeling) involves detection of DNA strand breaks by the enzymatic labeling of the 3'-OH termini with modified nucleotides. Detection by flow cytometry or microscopy.	Upon induction of apoptosis, rapid alterations of the PL's in most cell types occurs, leading to exposure of PS on the cell surface (flip-flop mechanism). High affinity binding of Annexin V to PS occurs in presence of Ca. Annexin V is conjugated to FITC or biotin for detection. Detection by flow cytometry and fluorescence microscopy.	Anti-PARP: DNA binding protein and repair enzyme that recognizes DNA strand breaks and is a substrate for caspase-3. Detection by Western blot Some methods allow for flow cytometry detection.	Caspase activity is measured using various methods. Detection can be measured using flow cytometry, microscopy, ELISA, or colorimetric methods.
Advantages	Detects accumulation of product over time. Global apoptosis assay. Detects early apoptosis. Apoptosis indicator / Predictive indicator. Useful tool to investigate tumor apoptosis for drug screening & pro-apoptotic compounds, dose-response curves, clinical research. Useful for cancer therapy research since CK18 not expressed in bone marrow. Only 96-well kit that can do high throughput drug screening and time-course kinetics. Fast, simple, robust, reproducible, convenient. Can measure time-course kinetics. M30 antibody works well on formalin-fixed, paraffin-embedded tissues. Will not detect necrotic or viable cells. Insensitive to incubation temp. or agitation speed. Freeze-thawing of samples is OK. Hemolyzed samples are OK.	Apoptosis morphology is easily detected. Works well on formalin-fixed, paraffin-embedded tissues. Applicable for many cell types and species.	Global apoptosis assay. Detects early apoptosis. Applicable for high throughput screening. Easy sample prep., Simple procedure. Applicable for many cell types and species. Fast and sensitive with short inc. times. Many kit options available.	Global apoptosis assay. Detects early apoptosis. Will not detect necrotic cells. Applicable for many cell types.	Global apoptosis assay. Detects early apoptosis. Will not detect necrotic cells. Applicable for many cell types. Method is generally convenient and sensitive. Some assays allow for frozen sample testing. Many kit options available.
Disadvantages	Only applicable to epithelial cells. Cannot use for non-caspase related apoptosis.	Detects late apoptosis. Can detect necrotic cells. Length of time tissues sit before fixation can affect assay. Section thickness can influence assay Certain amount of optimization is required.	Can detect necrotic cells. Too difficult to get time points for time-course kinetic measurements.	Less convenient than ELISA format Too difficult to get time points for time-course kinetic measurements.	Can be misleading due to varying time course kinetics. Cannot use for non-caspase related apoptosis. Must know which caspases are activated. Some methods require live cells.