Acute Phase
Apoptosis
Cancer
Cardiovascular Disease
Cytokines Chemokines, Growth Factors
Diabetes
Gene Expression
Genotyping
Immunoglobulin Isotyping
MicroRNA Expression

Bio-Plex Pro™ RBM Apoptosis Assays

MAGNETIC SEPARATION ENABLED

Bak, Bax, Lamin B, Smac, Bad, Bax/Bcl-2 Dimer, Bcl-xL, Bim, Mcl-1, Active Caspase-3, Bcl-xL/Bak Dimer, Mcl-1/Bak Dimer, and Survivin

- Magnetic workflow
- All-in-one kit format
- 2-level quality controls

High-Performance Multiplex Immunoassays for Apoptosis Research

The Bio-Plex Pro RBM apoptosis assays, developed in partnership with Myriad RBM, comprise a highly relevant set of intracellular proteins involved in the commitment, onset, and induction of **apoptosis by the intrinsic pathway** (Figure 1). Myriad's close collaboration with SAIC-Frederick, Inc (now Leidos Biomedical Research, Inc., which operates labs at NCI) was instrumental in the selection and clinical validation of markers found in these panels (Table 1). The assays are built on magnetic beads to enable robust quantification of multiple proteins in cell and tissue lysates. Assays are offered as premixed all-in-one kits for research involving:

- Cancer cell biology
- Mechanism of action of cancer drugs
- Neurodegenerative diseases (such as Alzheimer's or Parkinson's)
- Stroke
- Heart failure
- Viral infection (such as hepatitis)

Assay features

- Optimized for high precision and lot-to-lot reproducibility of sample measurements
- Magnetic beads for simplified plate processing
- 2-level quality controls with lot-specific ranges
- Assay quick guide to get you started right away
- Compatible with Bio-Plex® 200, Bio-Plex 3D, and Bio-Plex® MAGPIX™ systems

Assay Performance Definitions

The following parameters are indicative of assay performance, as shown in Table 2.

Assay working range — the range of concentrations within which the assay is precise and accurate. Boundaries of the assay working range are defined by the lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ)

Precision — the coefficient of variation (%CV) at concentrations within the assay working range

Accuracy (recovery) — percentage of the observed concentration relative to the expected concentration of a known amount of analyte within the assay working range

Sensitivity (limit of detection, LOD) — the concentration of analyte for which the fluorescence intensity signal is two standard deviations above the background signal

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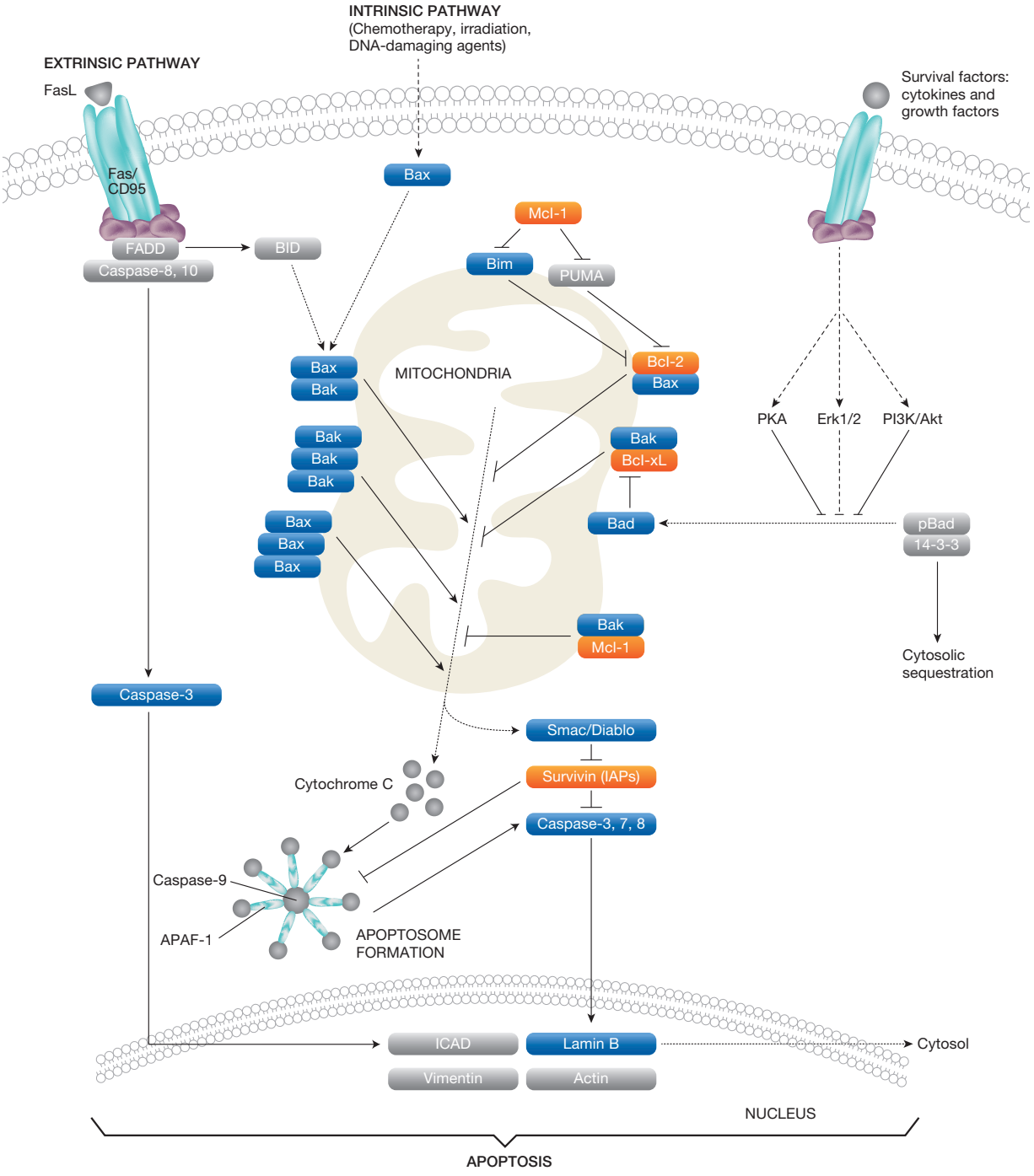

Fig. 1. Apoptosis is induced by at least two distinct signaling pathways, the extrinsic and intrinsic pathway. The extrinsic pathway is triggered by signaling through death receptors such as Fas, followed by downstream activation of caspase-8 and caspase-3. The intrinsic pathway is triggered by cytotoxic stress, which leads to translocation of Bcl-2 family proteins, Bax and Bak, to the mitochondrial membrane. Oligomerization of Bax and Bak causes release of cytochrome C into the cytosol, which promotes apoptosome formation, caspase activation, degradation of nuclear lamin B, and cell death. Pro-apoptotic proteins included in the panels (▬); anti-apoptotic proteins included in the panels (▬); proteins involved in apoptosis but not included in the panels (▬).

Table 1. Bio-Plex Pro RBM apoptosis analytes.

Short Name	Description	Apoptotic Action	Apoptotic Cell
Bak	**Bcl-homologous antagonist/killer** is a pro-apoptotic member of the Bcl-2 family. In healthy cells, it is integrated in the mitochondrial outer membrane. Upon apoptotic stimuli, Bak forms oligomer channels in the mitochondrial membrane for cytochrome C release. This activity is regulated by forming a complex with anti-apoptotic Mcl-1 and Bcl-xL.	Pro-apoptotic	Mitochondrial membrane
Bax	**Bcl-2 associated protein X** is a pro-apoptotic member of the Bcl-2 family. In healthy cells, it is found as a monomer in the cytosol, but upon apoptotic stimuli, it translocates to the mitochondrial outer membrane, and forms large oligomeric complexes. There it interacts with pore proteins to enable cytochrome C release into the cytosol, and initiate the caspase activation pathway for apoptosis. Bax may cycle to the mitochondrial membrane and dimerize with Bcl-xL.	Pro-apoptotic	Moves to the mitochondrial membrane
Lamin B, intact and 45 kD	Nuclear lamins are proteins of intermediate filament type, located at the outer rim of the nucleus. They consist of two types of polypeptides, lamin A and lamin B. Lamin B consists of B1 and B2 subtypes. Lamins mechanically stabilize the cell nucleus and also play a role in DNA replication and chromatin organization. Lamin B is cleaved by caspase-3 and caspase-6 during the early phases of apoptosis (up to 90 min) before DNA fragmentation. Detection of cytoplasmic dissociated lamin B indicates cell apoptosis.	Early indicator of apoptosis	Cytosol
Smac	**Second mitochondria-derived activator of caspase** is a dimeric mitochondrial protein synthesized in the cytoplasm as a 239 amino acid precursor protein, with 55 amino acids at the N-terminus serving as a mitochondrial-targeting sequence. Under apoptotic stimuli, it is proteolytically cleaved to a 23 kD active form and released into the cytosol together with cytochrome C, where it reverses IAP inhibition of caspase-9, allowing caspase-9 to activate the caspase cascade.	Pro-apoptotic	Cytosol
Bad	**Bcl-2-associated death** promoter is a pro-apoptotic, BH3-only–binding domain member of the Bcl-2 family. BH3-only proteins connect apoptotic death signals to the activation of Bax and Bak, which control mitochondrial membrane disruption and apoptosis. Phosphorylated Bad (pBad) is typically bound to the cytosolic protein 14-3-3, and is thus sequestered away from the mitochondria. Dephosphorylation results in the release of cytosolic (free) Bad, which binds to and inhibits the pro-survival activity of Bcl-2 family proteins at the mitochondrial membrane.	Pro-apoptotic	Cytosol (free)
Bax/Bcl-2 dimer	**B-cell lymphoma-2** is an anti-apoptotic protein that resides on the outer mitochondrial membrane. When bound to **Bax** as a heterodimer, it inhibits permeability of the mitochondrial membrane, preventing release of cytochrome C.	Anti-apoptotic	Mitochondrial membrane; decreased with apoptosis
Bcl-xL	**B-cell lymphoma-extra large** is an anti-apoptotic member of the Bcl-2 family found in the outer mitochondrial membrane. Heterodimerization with pro-apoptotic proteins (especially Bak) inhibits apoptosis by preventing release of cytochrome C.	Anti-apoptotic	Mitochondrial membrane
Bim	**Bcl-2-interacting mediator** of cell death is a pro-apoptotic protein belonging to the BH3-only group of the Bcl-2 family. Bim binds and antagonizes pro-survival members of the Bcl-2 family such as Mcl-1, Bcl-xL, and Bcl-2. Three prominent isoforms are generated by alternative splicing: Bim-S, Bim-L, and Bim-EL. Each isoform has the ability to induce apoptosis.	Pro-apoptotic	Cytosol or mitochodrial membrane if dimerized
Mcl-1	Induced **myeloid leukemia cell differentiation protein-1** is an anti-apoptotic member of the Bcl-2 family. Heterodimerization with pro-apoptotic proteins (especially Bak) inhibits apoptosis. While Mcl-1 may not be as potent a protector against apoptosis as Bcl-2, it does appear to be the main anti-apoptotic protein in some cell types including neutrophils.	Anti-apoptotic	Mainly mitochondrial membrane, some in cytosol
Active caspase-3	**Cysteinyl aspartyl protease-3** belongs to the peptidase C14A enzyme family and is known to play an important role in the apoptotic cascade. The active enzyme is formed by cleavage of the inactive 32 kD pro-enzyme into the p17 and p12 subunits. Two of each subunit noncovalently heterodimerize giving the final enzyme two catalytic sites. Active caspase-3 cleaves and activates other caspases and is a primary regulator of apoptotic-associated proteolysis.	Pro-apoptotic	Cytosol (high levels)
Bcl-xL/Bak dimer	Anti-apoptotic Bcl-xL heterodimerizes with Bak at the mitochondrial outer membrane and inhibits permeability of the mitochondrial membrane, preventing release of cytochrome C. During apoptosis BH3-only proteins, such as Bim and Bad, will bind to Bcl-xL and cause Bak to be released. Upon release, Bak will oligomerize creating pores in the mitochondrial membrane.	Anti-apoptotic	Mitochondrial membrane; decreased with apoptosis
Mcl-1/Bak dimer	Anti-apoptotic Mcl-1 heterodimerzes with Bax and Bak at the mitochondrial outer membrane to prevent their activation, thus inhibiting cytochrome C release from the mitochondria.	Anti-apoptotic	Mitochondria; decreased with apoptosis
Survivin	Survivin is a 16 kD member of the Inhibitor of Apoptosis (IAP) family which also plays a role in chromosome segregation and cytokinesis. The anti-apoptotic function of survivin comes from its ability to inhibit the activation of caspases-3 and -7. Survivin is found to be upregulated in various tumors.	Anti-apoptotic	Cytosol/nucleus

Table 2. Representative assay performance.

Target	Bead Region	Assay Working Range, ng/ml		Assay Sensitivity, ng/ml	Assay Precision	
		LLOQ	ULOQ	LOD	Intra-assay %CV	Inter-assay %CV
Panel 1						
Bak	74	0.43	630	0.18	6%	14%
Bax	27	0.25	255	0.26	10%	19%
Lamin B, intact and 45 kD	14	0.057	95	0.044	5%	14%
Smac	19	0.16	165	0.075	8%	19%
Panel 2						
Bad	73	0.27	200	0.18	5%	9%
Bax/Bcl-2 dimer	42	0.47	1020	0.47	7%	9%
Bcl-xL	22	0.070	30	0.046	4%	7%
Bim	12	0.014	16	0.015	4%	8%
Mcl-1	18	0.10	180	0.10	6%	6%
Panel 3						
Active caspase-3	57	0.039	50	0.023	6%	8%
Bcl-xL/Bak dimer	47	0.081	45	0.021	5%	14%
Mcl-1/Bak dimer	54	0.33	600	0.27	8%	13%
Survivin	20	0.056	50	0.023	6%	8%

The LLOQ, ULOQ, LOD, and inter-assay precision %CV are mean data determined from three independent multiplex assays. Intra-assay %CV is the mean of eight standard points run in triplicate within one representative assay. LLOQ and ULOQ are defined as the boundary standard curve points that meet precision and accuracy specifications of ≤30% intra-assay CV and 80–120% recovery. Data were generated using the magnetic workflow with the Bio-Plex Pro II wash station.

Accuracy and Sensitivity

Exceptional quality of the Bio-Plex Pro RBM apoptosis assays ensures high accuracy and sensitivity. The overall accuracy of the assays is provided by the standard curves generated in Bio-Plex Manager™ software. Standard curves were obtained for Bad, active caspase-3, and Smac (Figure 2). Sensitivity was analyzed by comparing total Bak and Bcl-xL assays with that of western blotting (Figure 3). Lower protein concentration levels of each apoptotic marker were detectable with the Bio-Plex Pro RBM apoptosis assays than with western blotting methods.

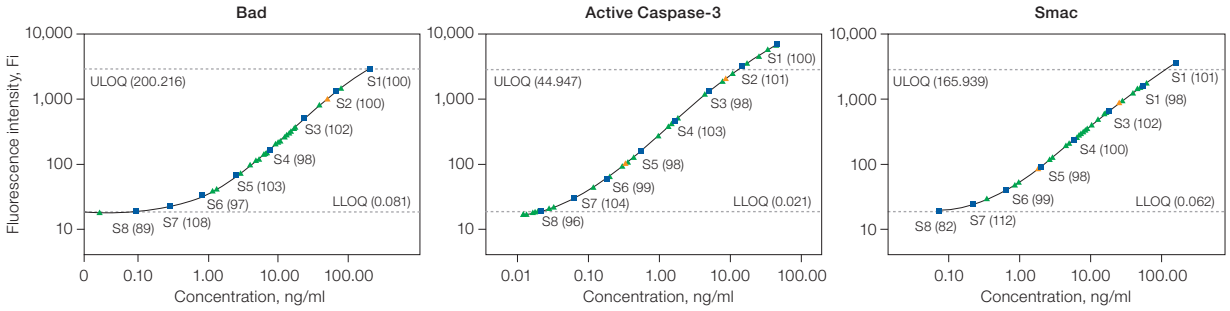

Fig. 2. Standard curves with assay controls and cell lysates. Standard points were prepared by serially diluting a reconstituted standard threefold to generate an eight-point standard curve. Standard points with % recovery (■); controls (▲); samples (▲). Data were generated in Bio-Plex Data Pro™ software.

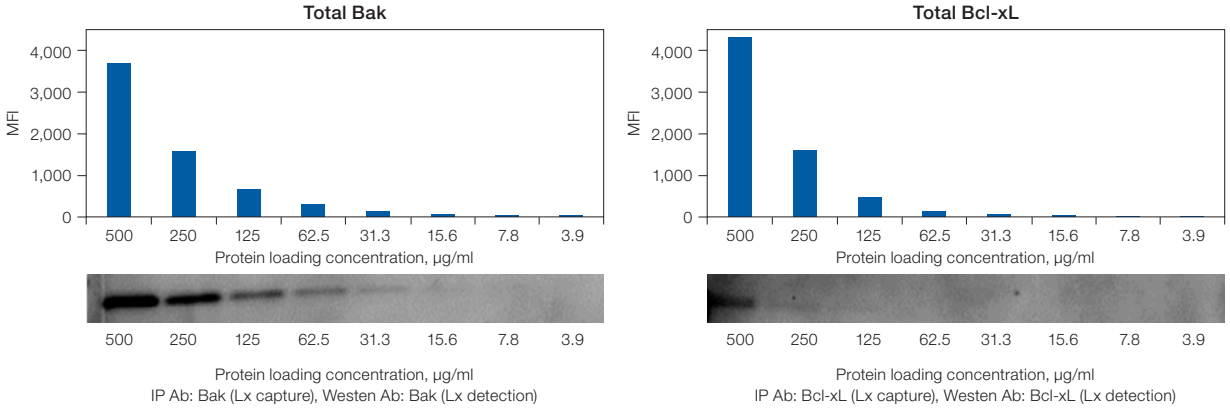

Fig. 3. Sensitivity of Bio-Plex Pro RBM apoptosis assay compared to western blotting. A lysate purified from the nuclear + mitochondrial fraction of an untreated PC3 xenograft cell line was serially diluted and then measured using the Bio-Plex Pro RBM apoptosis kit and western blotting methods. The Bio-Plex assays demonstrated superior sensitivity down to the single digit µg/ml protein loading.

Expression Pattern of Apoptotic Markers

Expression levels of pro-apoptotic and anti-apoptotic markers were established in healthy and cancer colon biopsies as well as tissue culture samples treated with the cancer drugs GSP, ABT, and HA14-1 (Figures 4 and 5).

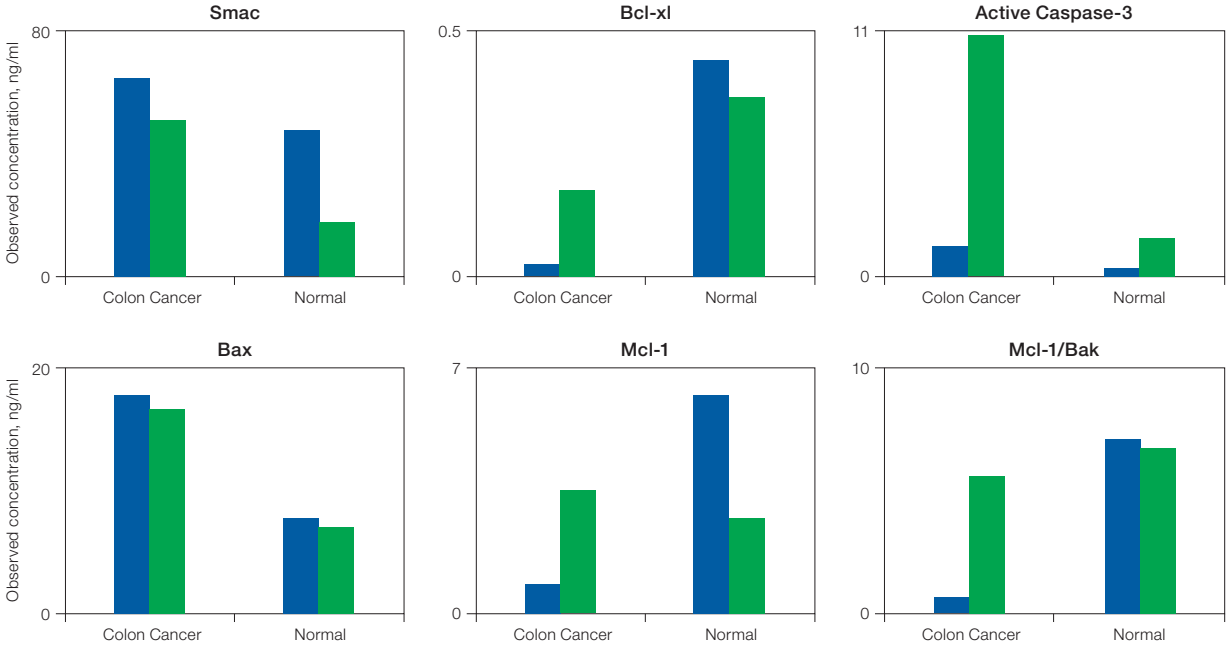

Fig. 4. Detection of apoptosis biomarkers in colon cancer tissue. A representative tissue was processed to compare the levels of apoptosis biomarkers in two sample fractions (nuclear + mitochondrial and cytosolic) relative to a non-matching normal colon tissue. Nuclear + mitochondrial (■); cytosolic (■).

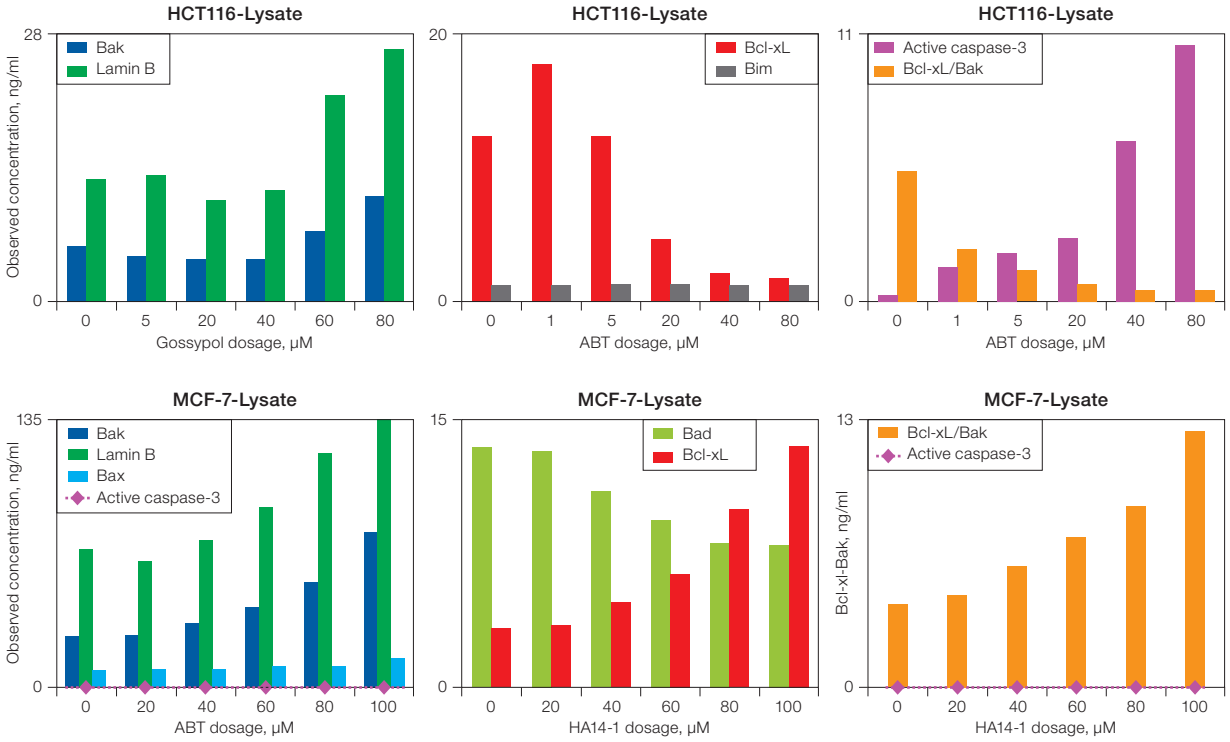

Fig. 5. Drug induced apoptosis. HCT-116 (ATCC® CCL-247™) and MCF7 (ATCC® HTB-22™) cells were treated for 3 hours with increasing concentrations of gossypol (0, 5, 20, 40, 60, and 80 µM), ABT-263 or HA14-1 (0, 20, 40, 60, 80 and 100 µM). Whole cell lysates were analyzed with the multiplex assays.

Ordering Information

Catalog #	Description
171-WAR1CK	**Bio-Plex Pro RBM Apoptosis Panel 1**, 1 x 96-well all-in-one kit that includes premixed magnetic capture beads and detection antibodies, standards, 2-level controls, standard diluent, buffers (blocking, lysate dilution (LDB), cytosolic extraction (CEB), 10x assay), 10x streptavidin-PE, flat bottom plate, plate seals, and instructions, for the detection of the following analytes in cell and tissue lysates: Bak, Bax, lamin B, and Smac
171-WAR2CK	**Bio-Plex Pro RBM Apoptosis Panel 2**, 1 x 96-well all-in-one kit that includes premixed magnetic capture beads and detection antibodies, standards, 2-level controls, standard diluent, buffers (blocking, lysate dilution (LDB), cytosolic extraction (CEB), 10x assay), 10x streptavidin-PE, flat bottom plate, plate seals, and instructions, for the detection of the following analytes in cell and tissue lysates: Bad, Bax/Bcl-2 dimer, Bcl-xL, Bim, and Mcl-1
171-WAR3CK	**Bio-Plex Pro RBM Apoptosis Panel 3**, 1 x 96-well all-in-one kit that includes premixed magnetic capture beads and detection antibodies, standards, 2-level controls, standard diluent, buffers (blocking, lysate dilution (LDB), cytosolic extraction (CEB), 10x assay), 10x streptavidin-PE, flat bottom plate, plate seals, and instructions, for the detection of the following analytes in cell and tissue lysates: Active caspase-3, Bcl-xL/Bak dimer, Mcl-1/Bak dimer, and survivin
Wash Stations and Accessories	
300-34376	**Bio-Plex Pro Wash Station**, microplate wash station for magnetic bead–based assays, includes magnetic plate carrier, waste bottle, 2 liquid bottles
171-020100	**Bio-Plex Handheld Magnetic Washer**, includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex magnetic assays
171-025001	**Bio-Plex Pro Flat Bottom Plates**, pkg of 40, 96-well plates, for use with Bio-Plex Pro wash stations when using magnetic bead–based assays
Software	
171-001510	**Bio-Plex Data Pro Software with Bio-Plex Manager Software**, Bio-Plex Data Pro software (5 seats), for multi-experiment analysis and advanced data visualization, and Bio-Plex Manager software (5 seats), for instrument data evaluation and optimization. CDs and security HASP key included
171-001513	**Bio-Plex Data Pro Software**, (5 seats), for multi experiment analysis and advanced data visualization
171-STND01	**Bio-Plex Manager Software**, includes 1 user desktop license, for analysis of Bio-Plex data and generation of protocols, does not operate the instrument

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