Store at -20° and 4°C

#25576

CellSimple™ Apoptosis Multiplex Bead Assay Kit

1 Kit (50 assays)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Components Ship As: 23515	Item #	Kit Quantity	Storage Temp.
10X Apoptosis 4-Plex Bead Cocktail	43494	1.2 mL	4°C
10X Apoptosis 4-Plex Detection Antibody Cocktail	58184	1.2 mL	4°C
10X Streptavidin-PE Secondary	60489	1.2 mL	4°C
Array Diluent Buffer		2 x 15 mL	4°C
20X Array Wash Buffer		15 mL	4°C

Components Ship Separately:	Item #	Kit Quantity	Storage Temp.
1X Cell Lysis Buffer	7018	2 x 30 mL	-20°C

Storage: All components in this kit are stable for at least 6 months when stored at the recommended temperature. Kit should be stored at 4°C with the exception of Lysis Buffer, which is stored at –20°C (packaged separately).

Background References:

(1) Boatright, K.M. and Salvesen, G.S. (2003) *Curr Opin Cell Biol* 15, 725-31.

(2) Cohen, G.M. (1997) *Biochem J* 326 (Pt 1), 1-16.

(3) Bratton, S.B. and Cohen, G.M. (2001) *Trends Pharmacol Sci* 22, 306-15.

(4) Green, D.R. and Reed, J.C. (1998) *Science* 281, 1309-12.

Species Cross-Reactivity: H

Description: The CellSimple™ Apoptosis Multiplex Bead Assay Kit is a four-plex bead-based antibody assay founded upon the sandwich immunoassay principle. The assay kit allows for the simultaneous detection of four signaling molecules that are involved in the regulation of stress and apoptosis. Each target specific capture antibody has been covalently linked to a specific sized magnetic bead. Each kit contains enough reagents to run 50 samples allowing the user to generate 200 data points. The sample is incubated with the multiplex bead cocktail followed by a biotinylated detection antibody cocktail. Streptavidin linked Phycoerythrin is then used as a reporter to visualize the bound detection antibody. Analysis is then done using the CellSimple™ Cell Analyzer with the Multiplex Bead Assay Application.

Background: Cell death can occur due to a variety of circumstances including nutrient deprivation, inability to generate or store the energy required for metabolic functions, or deleterious environment that causes irreparable damage. Cells integrate multiple signals from a variety of sources before following either pro- or anti-apoptotic pathways (1). These signals can often carry conflicting information. Assessing the net effect of these processes in cell populations can be achieved by monitoring changes in a number of key signaling components. The caspase-3 and caspase-7 proteases exert a pro-apoptotic function through cleavage of multiple cellular targets. Caspase-3 and caspase-7 are activated by cleavage at Asp175 and Asp198, respectively (2). PARP is a DNA repair and apoptosis enzyme that is inactivated by cleavage at Asp214 by caspase-3 or caspase-7 (3). Akt is activated by stimulation of growth-factor receptors and primarily promotes anabolic growth and survival signals via targeting its broad array of substrates. Akt phosphorylates Bad at Ser112 and inhibits its ability to induce apoptosis (4).

CellSimple™ Cell Analysis System: The CellSimple™ Cell Analyzer is a benchtop instrument that utilizes a disposable thin-film cassette and a combination of a 488 nm laser, two photomultiplier tubes (525/45 nm and 561 nm LP filters), Coulter Principle-based cell measurements, and on-board software to provide easy-to-run applications and data analysis. Data acquisition occurs within approximately 10 seconds per test. The instrument relies on disposable cassettes for sample handling, which alleviates the need for flow cell cleaning and fluidics maintenance and the instrument is small enough to be portable between the lab bench and the hood. Applications include quantitative assessments of cell viability, apoptosis, other labeled antibody markers and single and multiplexed bead-based assays for protein and cellular analysis.

Specificity/Sensitivity: CellSimple™ Apoptosis Multiplex Bead Assay Kit detects the target proteins Cleaved PARP (Asp214), Cleaved Caspase-3 (Asp175), Cleaved Caspase-7 (Asp198) and Phospho-Bad (Ser112). No significant cross-reactivity has been observed between targets. This kit is optimized for cell lysates diluted to a total protein concentration between 0.1 and 0.5 mg/mL (see kit protocol).

Bead	Target	Modification
1	Cleaved PARP	Asp214
2	Cleaved Caspase-3	Asp175
3	Cleaved Caspase-7	Asp198
4	Phospho-Bad	Ser112

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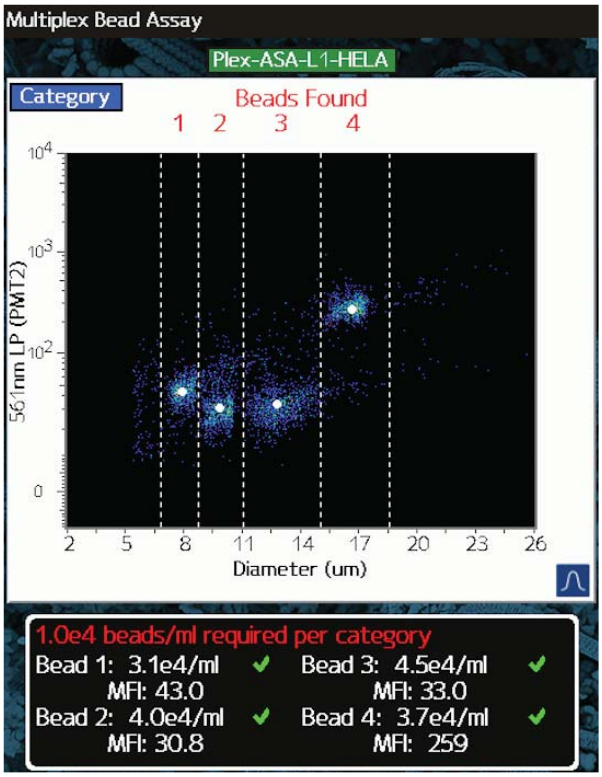
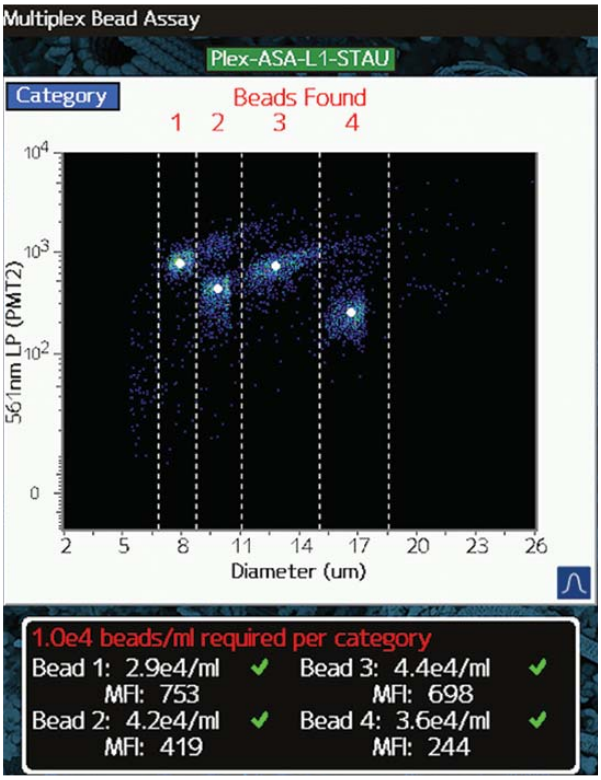
Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity: H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

HeLa Untreated



HeLa + Staurosporine



CellSimple™ bead-based analysis of untreated (left panel) and Staurosporine #9953 treated (1.0 µM, 3.5 hrs; right panel) HeLa cell lysates using the CellSimple™ Apoptosis Multiplex Bead Assay Kit. Data was collected in both the red channel (561 nm/LP) and bead size (µm) using the Multiplex Bead Assay application. Beads are analyzed and gated into 4 different groups according to size: Bead 1 - Cleaved PARP (Asp214), Bead 2 - Cleaved Caspase-3 (Asp175), Bead 3 - Cleaved Caspase-7 (Asp198), and Bead 4 - Phospho-Bad (Ser112). Median fluorescence intensity (MFI) along with bead concentrations are displayed in the rectangular box below the dot plot. Instrument screen shots are shown.

Plex-ASA-L1-STAU

Bead	(Plex-ASA-L1-H...) Reference MFI	(Plex-ASA-L1-S...) Unknown MFI	Ratio
1	43	753	17.50
2	31	419	13.57
3	33	698	21.12
4	259	244	0.94

The summary table shown here is generated from the Multiplex Bead Assay application after first analyzing the control or reference sample (untreated HeLa lysate) followed by the unknown or treated sample (HeLa + Staurosporine, 1.0 µM, 3.5 hrs) . The ratio between the unknown and reference sample is listed in the far right column for each target. Instrument screen shots are shown.

Please note your screen may look slightly different from the screen shots on the data sheet due to variations between software versions.

CellSimple™ Multiplex Bead Assay Procedure

A. Instrumentation: These kits were specifically designed for use with the CellSimple™ Cell Analyzer.

B. Kit components:

- 10X 4-Plex Bead Cocktail
- 10X 4-Plex Detection Antibody Cocktail
- 10X Streptavidin-PE Secondary
- Array Diluent Buffer
- 20X Array Wash Buffer
- 1X Cell Lysis Buffer (labeled on bottle as PathScan® Sandwich ELISA Lysis Buffer)

C. Additional reagents needed, but not supplied:

- 6-Tube Magnetic Separation Rack (#7017) or 12-Tube Magnetic Separation Rack (#14654)
- Protease/Phosphatase Inhibitor Cocktail (#5872)

D. Preparing Cell Lysates

Adherent cells

1. Thaw 1X Cell Lysis Buffer #7018 and mix thoroughly. Supplement Cell Lysis Buffer with a cocktail of protease inhibitors such as Protease/Phosphatase Inhibitor Cocktail (100X) #5872 (not included). Keep lysis buffer on ice.
2. Culture cells under conditions of interest and desired cell culture density. Treat cells with a modulator of interest (e.g. chemical compound, growth factor, cytokine or any combination of the above) for desired time.
3. Prior to cell lysis remove media and rinse cells once with ice-cold 1X PBS.
4. Aspirate / remove PBS and add 0.5 mL ice-cold 1X Cell Lysis Buffer (prepared in step 1) to each plate (10 cm diameter) and incubate the plate on ice for 5 minutes.
5. Scrape the cells off the plate and transfer to an appropriate tube. Keep on ice.
   Optional Step: Sonicate for 10–15 seconds to complete cell lysis and shear DNA (to reduce both sample viscosity and bead aggregation).
6. Microcentrifuge for 5 min. (~18,000 rcf) at 4°C and transfer the supernatant to a new tube. The supernatant contains the cell lysate. Store at -80°C in single use aliquots.

Suspension cells

1. Thaw 1X Cell Lysis Buffer and mix thoroughly. Supplement Cell Lysis Buffer with a cocktail of protease inhibitors such as the Protease/Phosphatase Inhibitor Cocktail. Keep lysis buffer on ice.
2. Collect cells by low speed (~130 rcf) centrifugation for 5 minutes and remove media. Suspend the cells with fresh medium to a cell density of 0.5-1.0 x 10^6 viable cells/mL. Treat cells with a modulator of interest (e.g. chemical compound, growth factor, cytokine or any combination of the above) for desired time.
3. Collect cells by low speed centrifugation (~130 rcf) and wash once with 5-10 mL ice-cold 1X PBS.
4. Lyse cells with 1X Cell Lysis Buffer as prepared in step 1. Roughly 2.0 mL of lysis buffer will be required to make cell extracts from 5.0 X 10^7 total cells (e.g. 50 mL of cell culture).
   Optional Step: Sonicate for 10–15 seconds to complete cell lysis and shear DNA (to reduce both sample viscosity and bead aggregation).
5. Microcentrifuge for 5 min. (~18,000 rcf) at 4°C and transfer the supernatant to a new tube. The supernatant contains the cell lysate. Store at -80°C in single use aliquots.

E. Assay Procedure (For Running 1 Sample)

1. Before use, bring the 1X Cell Lysis Buffer supplemented with protease inhibitors, 10X 4-Plex Bead Cocktail, 10X 4-Plex Detection Antibody Cocktail, 10X Streptavidin-PE Secondary, Array Diluent Buffer and the 20X Array Wash Buffer to room temperature.
2. Prepare the 1X Array Wash Buffer by diluting 20X Array Wash Buffer in deionized water. Dilute 15 mL of 20X Array Wash Buffer with 285mL of deionized water, label and keep at room temperature.
3. Prepare the 1X 4-Plex Detection Antibody Cocktail by diluting 21 µL of the 10X 4-Plex Detection Antibody Cocktail with 189 µL of Array Diluent Buffer. Keep the 1X Detection Antibody Cocktail on ice.
4. Prepare the 1X Streptavidin-PE Secondary by diluting 21 µL of the 10X Streptavidin-PE Secondary with 189 µL of Array Diluent Buffer. Keep the 1X Steptavidin-PE Secondary protected from light on ice.
5. Prepare the 1X 4-Plex Bead Cocktail solution. Vortex the 10X 4-Plex Bead Cocktail for 30 seconds. Add 20 µL of the 10X 4-Plex Bead Cocktail to 180 µL of 1X Cell Lysis Buffer into a labeled microcentrifuge tube. Vortex, then magnetically separate the beads using a magnetic separation rack. Allow the beads to separate for at least 1 minute then carefully aspirate the liquid using a pipette. Remove the tube from the magnetic separation rack then add 200 µL of 1X Cell Lysis Buffer to the beads. Vortex, then place the beads aside and proceed with the sample preparation steps.
   NOTE: We recommend using either the 6-Tube Magnetic Separation Rack #7017 or the 12-Tube Magnetic Separation Rack #14654.
6. Prepare the cell lysate sample by diluting the sample with 1X Cell Lysis Buffer to a working 1X concentration of 0.25 mg/mL.
   NOTE: The recommended final lysate protein concentration can range from 0.1 to 1.0 mg/mL.
   Optional Step: Sonicate the diluted lysate prior to adding to the washed beads to reduce aggregation.
7. Vortex and then magnetically separate the beads that were prepared as described within step 5. Carefully aspirate the liquid using a pipette.
8. Add 200 µL cell lysate to the beads, vortex, then place on a rotator and incubate for two hours at room temperature.
9. Spin down the microcentrifuge tube for 5 seconds using a mini-centrifuge followed by a brief vortex.
   NOTE: This step is to make sure all of the material has been spun down to the bottom of the microcentrifuge tube, followed by vortexing to break up any bead clumps that may have formed after centrifugation.
10. Magnetically separate the beads using a magnetic separation rack for 1 minute.
11. Carefully aspirate the liquid using a pipette.
12. Remove the microcentrifuge tube from the magnetic separation rack then wash the beads with 200 µL of 1X Array Wash Buffer.
13. Vortex the beads then place them back into the magnetic separation rack. Allow the beads to separate for 1 minute.
14. Carefully aspirate the liquid using a pipette.
15. Repeat wash steps 12 through 14.
16. Add 200 µL of 1X Detection Antibody Cocktail to the beads, vortex, then place on a rotator and incubate for one hour at room temperature.
17. Repeat wash steps 9 through 14 twice for a total of 2 washes.
18. Add 200 µL of 1X Streptavidin-PE secondary to the beads, vortex, then place on a rotator and incubate for 30 minutes at room temperature protected from light.
19. Repeat wash steps 9 through 14 twice for a total of 2 washes.
20. Add 200 µL of 1X Array Wash Buffer then vortex. The sample is now ready to be analyzed on the CellSimple™ Cell Analyzer.
21. Analyze 75 µL samples with the CellSimple™ Cell Analyzer using the Multiplex Bead Assay Application. Please see the CellSimple™ User Guide for more details about using the Multiplex Bead Assay Application..