Revision 1

Store at -20C

#7101

PathScan® Multi-Target HCA DNA Damage Kit

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Kit Includes*	Quantity	Applications	Dilution	Isotype
Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-Chk1 (Ser345) (133D3) Rabbit mAb	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-Chk2 (Thr68) Antibody	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-p53 (Ser15) (16G8) Mouse mAb	140 µl	HCA, ICW, IF-IC	1:10	Mouse IgG1
p21 Waf1/Cip1 (12D1) Rabbit mAb	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-cdc2 (Tyr15) (10A11) Rabbit mAb	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-p38 MAPK (Thr180/Tyr182) (D3F9) XP™ Rabbit mAb	140 µl	HCA, ICW, IF-IC	1:10	Rabbit IgG
Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb	140 µl	HCA, ICW, IF-IC	1:10	Mouse IgG1

*Component formulation specific to kit.

Applications Key: HCA=High Content Analysis, ICW=In-Cell Western, IF-IC=Immunofluorescence (Immunocytochemistry)

Description

CST's PathScan® Multi-Target HCA DNA Damage Kit contains eight primary antibodies that target the DNA damage cellular signaling pathway. This kit is designed to elucidate the signaling occurring through key pathway nodes using automated imaging or laser scanning platforms or manual immunofluorescent microscopy. The kit provides the investigator with a quick and easy means to choose the endpoints that will be the most robust and useful for subsequent studies, whether large high content/high throughput screening projects or single small-scale experiments. The antibodies are supplied at 10X of their optimal dilution for immunofluorescent applications. This allows the antibodies to be easily diluted to their 1X working concentrations and dispensed into multi-well plates or slides. 140 µl of each antibody is supplied, which is sufficient for 24 wells on 96-well plates (50 µl 1X per well) or one row on two 96-well plates.

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Background

Cell cycle control involves an ordered series of cellular signaling events designed to maintain the integrity and proper function of cells and biological pathways. Cells have evolved complex mechanisms, collectively termed the DNA damage checkpoint, to modulate cell cycle progression in response to genomic insult. Damage to DNA caused by either internal or external sources such as UV light, ionizing radiation, genotoxic agents, etc. initiates a cascade of events that blocks cell cycle progression to either allow time to repair damaged DNA, or activate cell death pathways if too much damage has been incurred. Monitoring the signaling components of the DNA damage pathway is important to aid in understanding the aberrant cellular signaling associated with unchecked cell cycle control in disease states such as cancer. Cell cycle progression can be affected by the DNA damage checkpoint at the G1/S transition, during S phase, or at the G2/M phase transition. At G2/M, cdc2/cyclin B activity acts as a master regulator of entry into mitosis (1). The cdc25C phosphatase removes inhibitory phosphorylation on Thr14 and Tyr15 of cdc2, allowing for maximal activation of cdc2/cyclin B (1,2). When DNA damage occurs, a signaling cascade is activated that inhibits the ability of cdc25 to activate cdc2/cyclin B. A proximal event in the signaling pathway is localization of Ser139-phosphorylated histone H2A.X to sites of DNA damage at subnuclear foci (3). Histone H2A.X and other mediators recruit additional signaling molecules to the site of the damage, activating the ATM/ATR kinases, the central mediators of the DNA damage response. ATM is primarily activated by double strand breaks of DNA (4,5), while ATR is activated by a variety of DNA lesions and replication stresses (6). ATM/ATR in turn initiate two parallel cascades that inactivate the cdc2/cyclin B complex (7). The first cascade rapidly inhibits progression into mitosis through the activation of the Chk kinases (Chk1 for ATR and Chk2 for ATM), which

phosphorylate and inactivate cdc25, preventing activation of cdc2/cyclin B (8-10). The more long-term second cascade involves phosphorylation of the tumor suppressor protein p53, leading to either cell cycle arrest and DNA repair or apoptosis through regulation of p53 downstream effectors, including the tumor suppressor protein p21 Waf1/Cip1 (11). Upon DNA damage, p53 is phosphorylated at a number of sites and up-regulates p21 transcription via a p53 responsive element. p21 expression can block cell cycle progression by inhibiting a subset of the cyclin-dependent kinases including cdc2 (12,13). In addition to canonical ATM/ATR checkpoint signaling, the SAPK/JNK and p38 MAP kinase pathways are activated by a variety of cellular stresses including inflammatory cytokines, UV light, and growth factors (14,15). These stress-activated pathways contribute to G2/M checkpoint control through activation of p53 and other MAPK substrates, such as MAPKAPK-2, which can directly affect components of the checkpoint cascade, such as cdc25 (8).

Background References

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