abcam

Product datasheet

Human p53 Total + pSer46 In-Cell ELISA Kit (IR) ab128570

3 Images

Overview

Product name Human p53 Total + pSer46 In-Cell ELISA Kit (IR)

Detection method IR

Precision

Intra-assay

Sample	n	Mean	SD	CV%
MCF-7 cells				< 10%

Sample type Adherent cells, Suspension cells

Assay type Cell-based (quantitative)

Assay duration Multiple steps standard assay

Species reactivity **Reacts with:** Human

Product overview p53 (TP53 gene) acts as a tumor suppressor in many tumor types and induces growth arrest or apoptosis depending on the physiological circumstances and cell type. p53 is involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. p53 mediated apoptosis induction seems to be by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. p53 is also implicated in Notch signaling cross-over.

In-Cell ELISA (ICE) technology is used to perform quantitative immunocytochemistry of cultured cells with a near-infrared fluorescent dye-labeled detector antibody. The technique generates quantitative data with specificity similar to Western blotting, but with much greater quantitative precision and higher throughput due to the greater dynamic range and linearity of direct fluorescence detection and the ability to run 96 samples in parallel. Because the p53 antibody is a mouse antibody and the p53 phospho S46 antibody is a rabbit antibody, their targets can be measured simultaneously in a same well using the cocktail of provided primary antibodies and the provided cocktail of IRDye®-labeled species-specific secondary antibodies when using a LI-COR infrared imager. This method rapidly fixes the cells in situ, stabilizing the in vivo levels of proteins and their post-translational modifications, and thus essentially eliminates changes during sample handling, such as preparation of protein extracts. Finally, the p53 phospho Ser46 and total p53 protein signals can all be normalized to cell amount, measured by the provided Janus Green

whole cell stain, to further increase the assay precision.

Plates are available in our ICE (In-Cell ELISA) Support Pack (**ab111542**) which can be bought seperately.

Tested applications **Suitable for:** In-Cell ELISA

Platform Microplate

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	
10X Phosphate Buffered Saline	1 x 100ml
100X Triton X-100	1 x 0.5ml
400X Tween-20	1 x 2ml
1000X IRDye® Labeled Secondary Antibody Cocktail	1 x 24µl
100X Mouse Anti-p53 Primary Antibody	1 x 120µl
100X Rabbit Anti-p53 Phospho Ser-46 Primary Antibody	1 x 120µl

Relevance Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. Implicated in Notch signaling cross-over. Prevents CDK7 kinase activity when associated to CAK complex in response to DNA damage, thus stopping cell cycle progression. Isoform 2 enhances the transactivation activity of isoform 1 from some but not all TP53-inducible promoters. Isoform 4 suppresses transactivation activity and impairs growth suppression mediated by isoform 1. Isoform 7 inhibits isoform 1-mediated apoptosis.

Cellular localization Cytoplasm. Nucleus. Nucleus › PML body. Endoplasmic reticulum. Note: Interaction with BANP promotes nuclear localization. Recruited into PML bodies together with CHEK2.

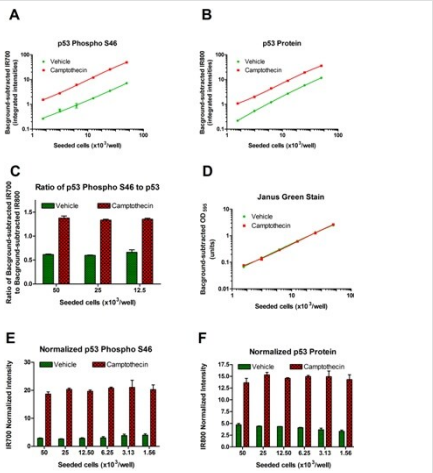
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab128570 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

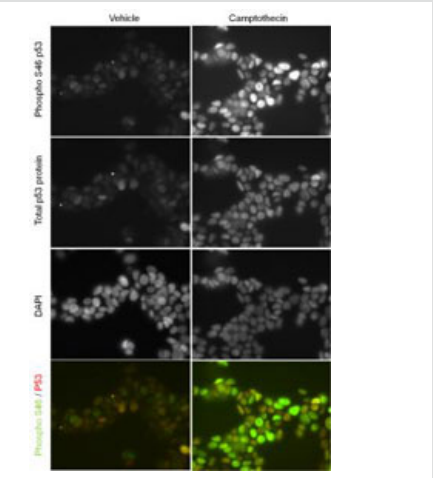
Application	Abreviews	Notes
In-Cell ELISA		Use at an assay dependent concentration.

Images



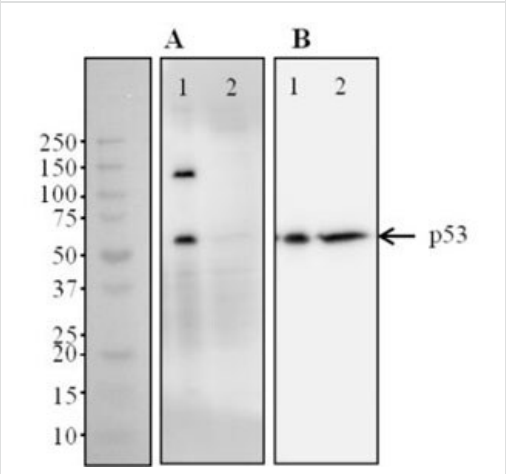
In-Cell ELISA - p53 Total + pSer46 Human In-Cell ELISA Kit (IR) (ab128570)

Sample experiment using ab128570 on MCF-7 cells treated with for 6 hours with Camptothecin or drug vehicle (DMSO). The data (presented as mean +/- SEM) were analyzed as described in Data Analysis section.



Immunocytochemistry/ Immunofluorescence - p53 Total + Phospho Ser46 Human Elisa Kit (ab128570)

Specificity of antibodies demonstrated by immuno-cytochemistry. Primary antibodies used in this assay kit were validated by staining MCF-7 cells treated with Camptothecin or vehicle and imaged by fluorescent microscopy. An increase in both p53 phospho S46 and total p53 protein signals upon Camptothecin treatment.



Western blot - p53 Total + Phospho Ser46 Human Elisa Kit (ab128570)

Antibody specificity demonstrated by Western Blot Analysis.
Primary antibodies used in this assay kit were validated by Western Blotting using Hek293T induced with Etoposide (to increase the levels of p53 phosphorylation). Cell extract was treated with λ protein phosphatase (lane 2) or left untreated (lane 1). Samples were analyzed by Western blotting using the rabbit p53 phospho S46 antibody (A). The membrane was re-probed with the mouse total p53 protein antibody (B). Note the sensitivity of the p53 phospho S46 signal to treatment with λ protein phosphatase and resistance of the total p53 protein signal to this treatment.

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