abcam

Product datasheet

Cell Cycle In-Cell ELISA Kit (Fluorescent) ab140363

5 References 3 图像

概述

产品名称	Cell Cycle In-Cell ELISA试剂盒(Fluorescent)
检测方法	Fluorescent
样品类型	Adherent cells
检测类型	Cell-based (quantitative)
检测时间	6h 30m
实验步骤	Multiple steps standard assay
种属反应性	**与反应:** Mouse, Human
产品概述	ab140363 is an In-Cell ELISA (ICE) assay kit that uses quantitative immunocytochemistry to measure levels of Cdk2 protein phosphorylated Tyr15 and Histone H3 protein phosphorylated Ser10 levels in cultured cells. Cells are fixed in a microplate and targets of interest are detected with highly specific, well-characterized antibodies. Relative target levels are quantified using secondary antibodies conjugated to either horseradish peroxidase (HRP) or alkaline phosphatase (AP) which generate signal through the use of two spectrally distinct fluorogenic substrates. Fluorescence is measured using a standard fluorescent spectrophotometer and relative levels of target proteins are quantified. Optionally, antibody signal intensity can be normalized to the total cell amount using Janus Green stain. In-Cell ELISA (ICE) 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 fluorescence detection and the ability to run up to 96 samples in parallel. 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. Plates are available in our ICE (In-Cell ELISA) Support Pack (**ab111542**) which can be bought seperately.
说明	The Cdk2 (pTyr15) + Histone H3 (pSer10) In-Cell ELISA Kit (Fluorescent) (ab140363) is designed to study cell cycle effects in response to various stimuli. Monoclonal antibodies specific to Cdk2 (pTyr15) and Histone H3 (pSer10) are used in this high-throughput duplexing plate-based assay. Cdk2 (pTyr15) is elevated in G1/S phase of the cell cycle and Histone H3 (pSer10) is elevated in G2/M phase. Cyclin-dependent kinase 2 (Cdk2) is a nuclear protein kinase that functions in the G1/S phase of the cell cycle. Inhibitory phosphorylation occurs on residues Thr14 and Tyr15; activation of Cdk2 includes dephosphorylation of these residues by cdc25. Cdk2 can form a complex with Cyclin A,

D or E. Phosphorylation of Cdk2 at Tyr15 indicates that a cell is at the G1/S transition.
Histone H3 is one of the four core histone proteins (H2A, H2B, H3 and H4) that pack DNA in nucleosomes. Post-translational modifications of histones include phosphorylation and acetylation and are important for chromatin assembly and gene expression. Phosphorylation of Histone H3 at Ser10 is tightly correlated with chromosome condensation during mitosis. Hence, Histone H3 pSer10 signal indicates a mitotic cell with condensed DNA.
In-Cell ELISA (ICE) technology is used to perform quantitative immunocytochemistry of cultured cells using enzyme linked secondary antibodies and fluorogenic substrates. 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 fluorescence detection and the ability to run up to 96 samples in parallel. Because the Cdk2 (pTyr15) antibody is a rabbit antibody and the Histone H3 (pSer10) antibody is a mouse antibody, they can be measured simultaneously in the same well using the cocktail of provided primary antibodies, species-specific secondary antibodies and fluorogenic substrates. This method rapidly fixes the cells in situ, stabilizing the in vivo levels of proteins and their post-translational modifications, and thus eliminating changes during sample handling, such as in the preparation of protein extracts. Finally, the Cdk2 (pTyr15) and Histone H3 (pSer10) signals can be normalized to cell amount, measured by the provided Janus Green whole cell stain, to further increase the assay precision.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

平台 Microplate

性能

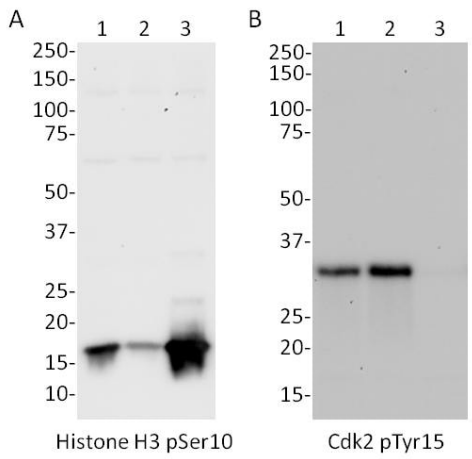
存放说明 Store at +4°C. Please refer to protocols.

组件	1 x 96 tests
1000X Anti-Mouse IgG/AP-Labeled Secondary Antibody	1 x 20µl
1000X Anti-Rabbit IgG/HRP-Labeled Secondary Antibody	1 x 20µl
100X Anti- Histone H3 (pSer10) Primary Antibody	1 x 120µl
100X Anti-Cdk2 (pTyr15) Primary Antibody	1 x 120µl
100X Triton X-100	1 x 500µl
10X Blocking Solution	1 x 10ml
10X Phosphate Buffered Saline	1 x 100ml
10X Quenching Solution	1 x 1.5ml
400X Fluorescent Substrate Cocktail	1 x 50µl
400X Tween-20	1 x 2ml

组件	1 x 96 tests
8000X Hydrogen Peroxide	1 x 50µl
Fluorescent Substrate Buffer	1 x 12ml
1X Janus Green Stain	1 x 17ml

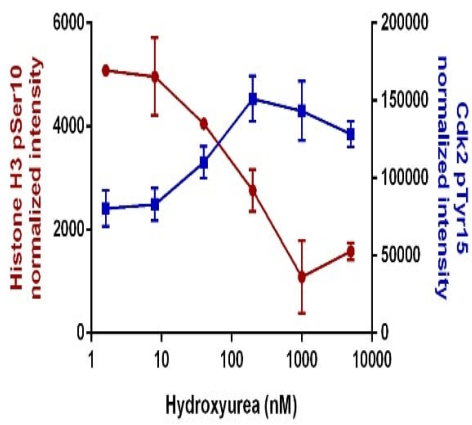
细胞定位 Cytoplasmic and Nuclear

图片



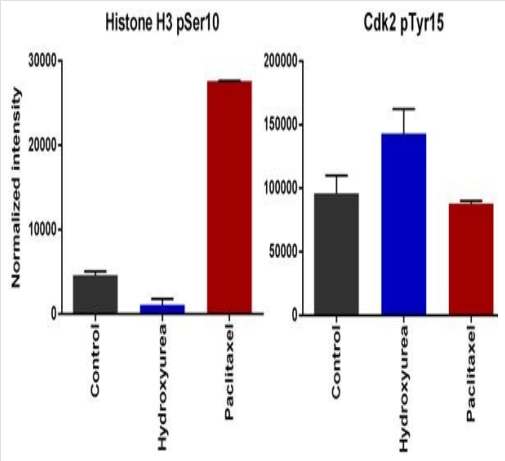
Antibody specificity demonstrated by Western Blot Analysis.

Whole cell lysates from HeLa cells were analyzed by Western blot with the primary antibodies used in this assay kit.
(A) Histone H3 pSer10 antibody:
Lane 1 -Untreated,
Lane 2- hydroxyurea = G1/S arrest,
Lane 3- paclitaxel = G2/M arrest .
(B) Cdk2 pTyr15 antibody:
Lane 1 -Untreated,
Lane 2-, thymidine = G1/S arrest,
Lane 3- nocodazole = G2/M arrest.



Sample data using ab140363 on HeLa cells treated with a titration of Hydroxyurea.

Data shown is for 24 hour treatment with 1.6 – 5000 nM hydroxyurea. Cdk2 pTyr15 intensity increases with hydroxyurea treatment dose whereas Histone H3 pSer10 intensity decreases.



Sample experiment using ab140363 on HeLa cells treated with Paclitaxel and Hydroxyurea.

Data shown is for 24 hour treatment with 1 mM hydroxyurea, 333 nM paclitaxel and untreated (Control). (Normalized intensity is described in section 9.)

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