abcam

Product datasheet

Cell Cycle (pCdk/pHH3/Actin) WB Cocktail ab136810

12 References 3 Images

Overview

Product name Cell Cycle (pCdk/pHH3/Actin) WB Cocktail

Species reactivity **Reacts with:** Mouse, Human

Product overview The Cell Cycle western blot cocktail (ab136810) is designed to provide a rapid assessment of cell cycle distribution based on molecular markers for the G1/S and M phases of the cell cycle. This is a mixture of three specific rabbit monoclonal primary antibodies targeting phospho-cdk2 Tyr15, phospho Histone H3 Ser10 and beta-actin. Cyclin-dependent kinase 2 (Cdk2) is maintained in an inactive state in G1/S by inhibitory phosphorylation on Tyr15. Histone H3 is phosphorylated at Ser10 when chromosomes condense during mitosis. Hence, elevated phospho-cdk2 Tyr15 indicates G1/S arrested cells and elevated phospho Histone H3 Ser10 indicates M-phase arrested cells when compared to asynchronous cycling control cells. An anti-Actin antibody is included as a loading control. These three readouts are easily resolved by western blot given their different molecular weights.

The recommended dilution for this cocktail is 1:250.

Tested applications **Suitable for:** WB

Properties

Storage instructions Store at -20°C. Please refer to protocols.

Components	200 µl
250X Cell Cycle WB Cocktail	1 x 200µl

Applications

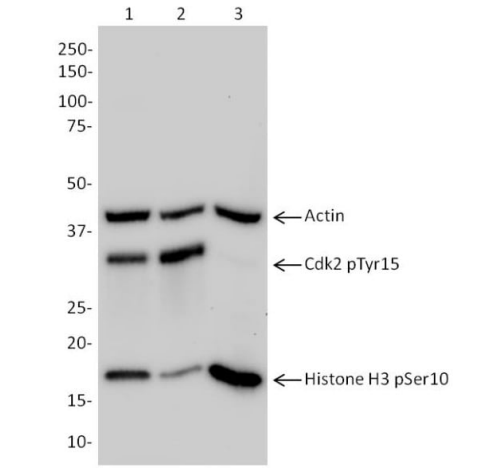
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab136810 in the following tested applications.

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

Application	Abreviews	Notes

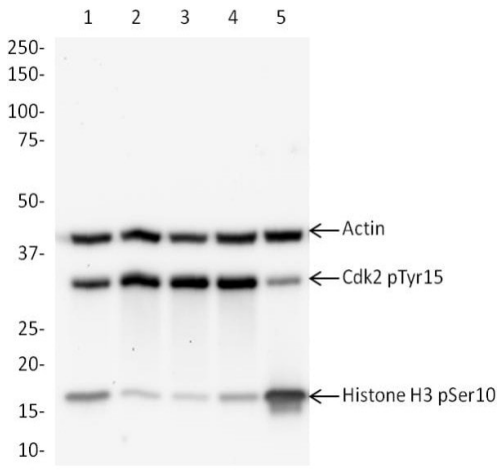
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. 1/250 dilution for primary antibody cocktail Suggested dilution buffer: 5% milk/PBS

Images



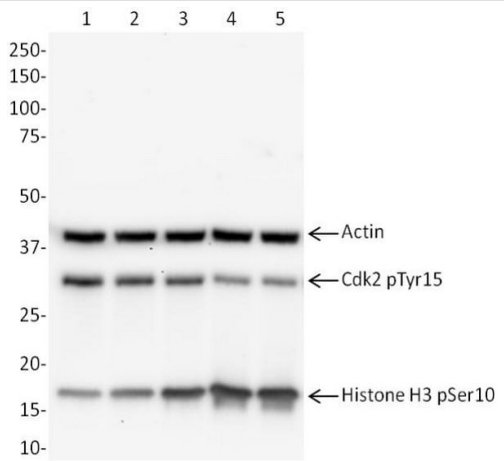
Western blot – ab136810 Cell Cycle Western Blot Cocktail

Western blot – ab136810 Cell Cycle Western Blot Cocktail Cdk2 pTyr15 and Histone H3 pSer10 bands are present in asynchronous cells. Cdk2 pTyr15 is elevated in G1/S arrested cells. Conversely, Histone H3 pSer10 is elevated in G2/M arrested cells. Primary antibody: All lanes Cell 250X Cell Cycle WB Cocktail diluted to 1X in 4% milk/PBS. Lane 1: HeLa lysate; Untreated, asynchronous cells Lane 2: HeLa lysate; G1/S arrested cells (thymidine treatment) Lane 3: HeLa lysate; G2/M arrested cells (sequential thymidine and nocodazole treatments) All lysates at 15 µg per lane. Lysates are **ab136811** HeLa Cell Cycle Lysates Secondary antibody: All lanes anti-rabbit-HRP. Predicted band size: 42, 33, 17 kDa.



Western blot – ab136810 Cell Cycle Western Blot Cocktail

Western blot – ab136810 Cell Cycle Western Blot Cocktail Hydroxyurea, Camptothecin and Etoposide treatments cause a G1/S-like arrest as judged by the increase in Cdk2 pTyr15 and decrease in Histone H3 pSer10 bands relative to control. In contrast, Paclitaxel causes a G2/M like arrest judged by the decreased Cdk2 pTyr15 and increased Histone H3 pSer10 bands. Primary antibody: All lanes Cell 250X Cell Cycle WB Cocktail diluted to 1X in 4% milk/PBS. Lane 1: HeLa lysate; Control treatment Lane 2: HeLa lysate; 5 mM Hydroxyurea, 24 h treatment Lane 3: HeLa lysate; 5 µM Camptothecin, 24 h treatment Lane 4: HeLa lysate; 1.5 µM Etoposide, 24 h treatment Lane 5: HeLa lysate; 2000 nM Paclitaxel, 24 h treatment All lysates at 15 µg per lane. Secondary antibody: All lanes anti-rabbit-HRP. Predicted band size: 42, 33, 17 kDa.



Western blot – ab136810 Cell Cycle Western Blot Cocktail

Western blot – ab136810 Cell Cycle Western Blot Cocktail Cdk2 pTyr15 intensity decreases and Histone H3 pSer10 intensity increases as a function of Paclitaxel concentration. Equivalent protein loading is indicated by the equal actin intensity across samples. Primary antibody: All lanes Cell 250X Cell Cycle WB Cocktail diluted to 1X in 4% milk/PBS. Lane 1: HeLa lysate; Control treatment Lane 2: HeLa lysate; 0.25 nM Paclitaxel, 24 h treatment Lane 3: HeLa lysate; 5 nM Paclitaxel, 24 h treatment Lane 4: HeLa lysate; 100 nM Paclitaxel, 24 h treatment Lane 5: HeLa lysate; 2000 nM Paclitaxel, 24 h treatment All lysates at 15 µg per lane. Secondary antibody: All lanes anti-rabbit-HRP. Predicted band size: 42, 33, 17 kDa.

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