Monitoring cell cycle processes critical to cancer development via parallel immunoassays.

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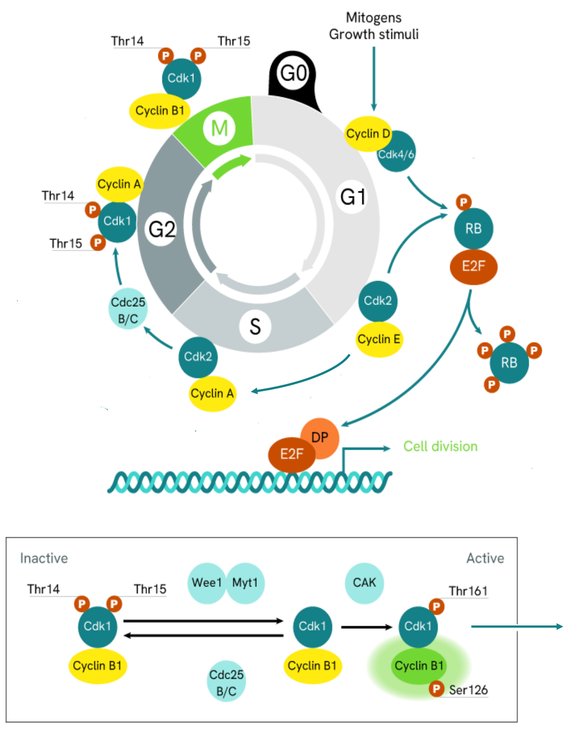
Revvity

1 Introduction

One key aspect of cell cycle regulation involves the nuclear protein Lamin A/C. Lamin A/C plays significant roles in chromosome organization, transcriptional regulation, DNA repair, cell signaling, and cell cycle regulation. Its phosphorylation is vital for nuclear lamina incorporation and disassembly during mitosis. Phosphorylation at "mitotic sites" (Ser22 and Ser392) peaks at mitosis onset, triggered by the CDK1-Cyclin B1 complex. These phosphorylation/dephosphorylation events within the CDK1-Cyclin B1-Lamin A/C pathway impact cell cycle alterations, affecting cell morphology, structure, and fate. Dysregulation of these phosphorylated proteins and their total counterparts is linked to increased cell growth, genomic instability, and cell senescence in various cancers, making it a key focus for developing effective therapies to halt tumor progression.

AlphaLISA™ SureFire™ Ultra™ technology offers a rapid and reliable method for tracking and analysis of cell cycle changes and cellular homeostasis. With unmatched sensitivity, it detects cellular events even in low cell numbers, surpassing other immunoassay techniques.

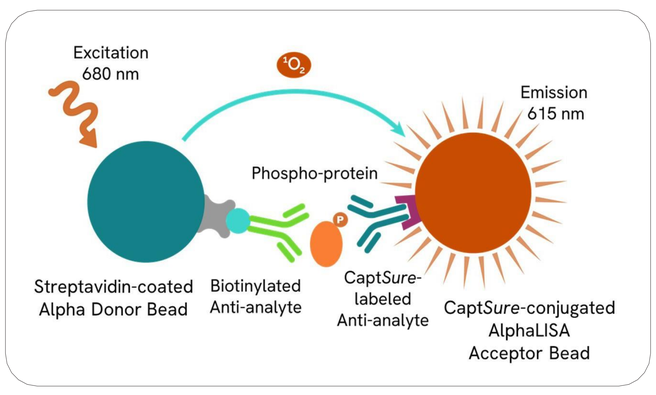
This poster showcases the exceptional performance of the AlphaLISA™ SureFire™ Ultra™ platform in monitoring phosphorylated and expression level of cell cycle regulatory proteins from the same cell lysate. Highlighting its utility in drug discovery and beyond, AlphaLISA™ SureFire™ Ultra™ technology is a powerful tool for unraveling the complexities of cell biology and the intricacies of the cell cycle.



2 Assay principle

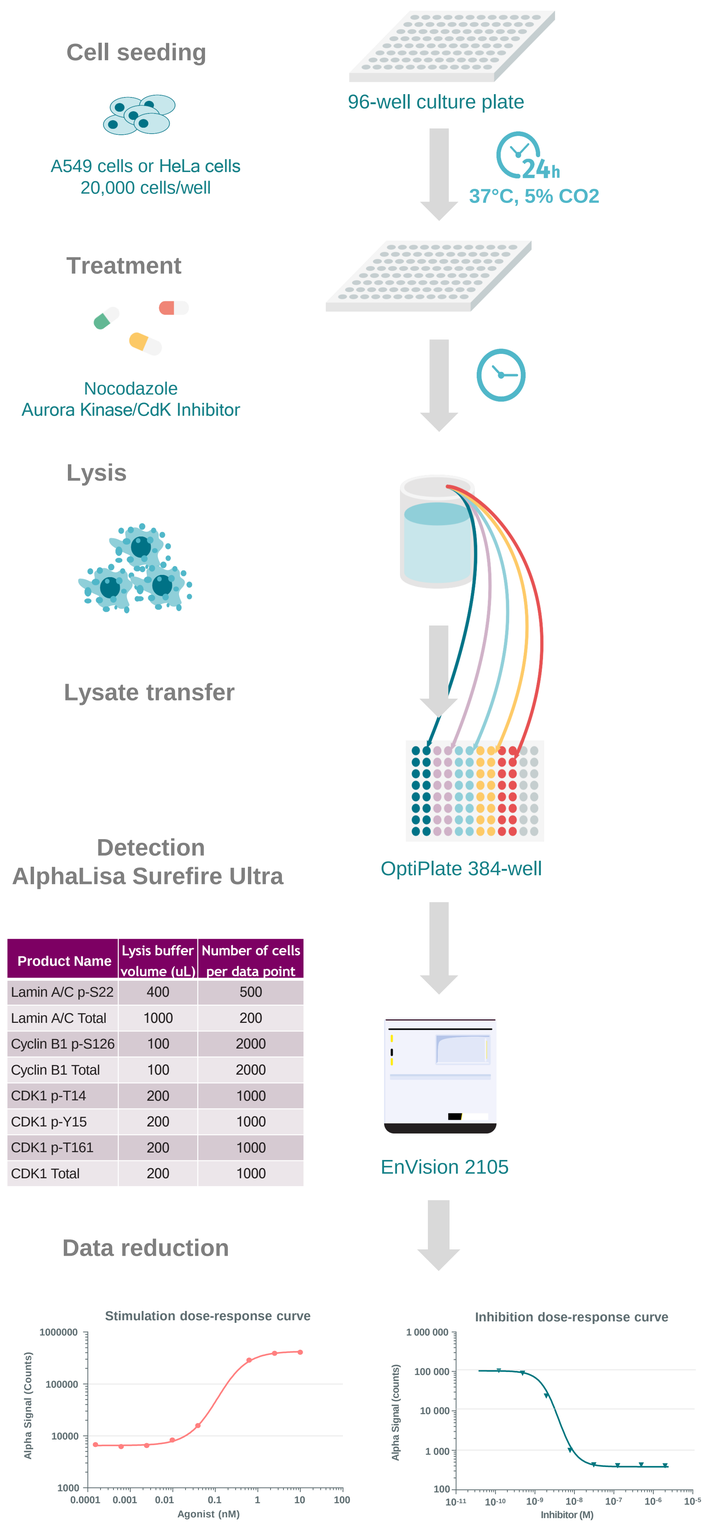
AlphaLISA technology is a fast, highly sensitive, homogeneous, no-wash bead-based assay platform that can be performed in a microplate format.
In the AlphaLISA™ SureFire™ Ultra™ assay, Donor beads are coated with streptavidin to capture one of the detection antibodies, which is biotinylated. Acceptor beads are coated with a proprietary CaptSure™ agent that immobilizes the other antibody, labeled with a CaptSure™ tag.

In the presence of target protein, the two antibodies bring the Donor and Acceptor beads close together. Upon excitation at 680 nm, an excited singlet oxygen diffuses and produces a chemiluminescent reaction in the Acceptor bead, leading to light emission at 615 nm while little to no signal over background is detected if Donor and Acceptor bead are not in proximity.
AlphaLISA™ signal is measured on Alpha compatible multimode plate reader, here the EnVision 2105 Multimode Plate Reader using AlphaLISA default settings (excitation at 680 nm and emission at 615 nm).



The results presented here represent the mean ± SD for triplicate samples and are representative of three independent experiments. Data was analysed in GraphPad Prism using a non-linear four parameter logistic regression for the dose-response curves.

3 Experimental workflow

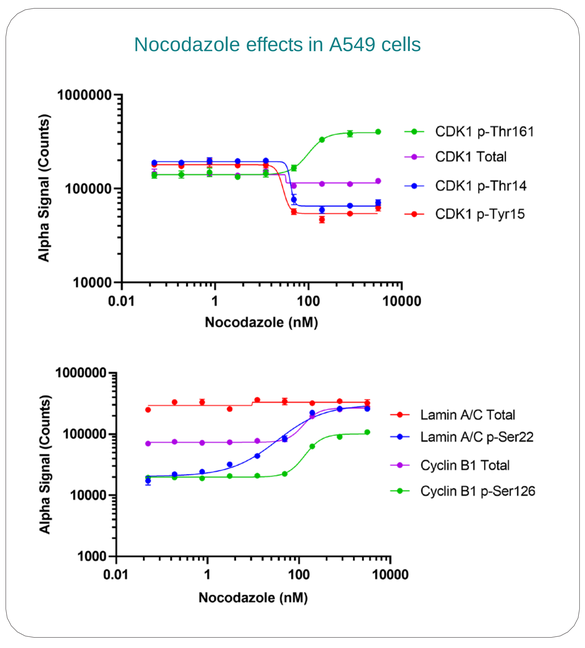


Product Name	Lysis buffer volume (uL)	Number of cells per data point
Lamin A/C p-S22	400	500
Lamin A/C Total	1000	200
Cyclin B1 p-S126	100	2000
Cyclin B1 Total	100	2000
CDK1 p-T14	200	1000
CDK1 p-Y15	200	1000
CDK1 p-T161	200	1000
CDK1 Total	200	1000

4 Results

Activation of CDK1 and Cyclin B1 triggers upregulation of Lamin A/C phosphorylation.

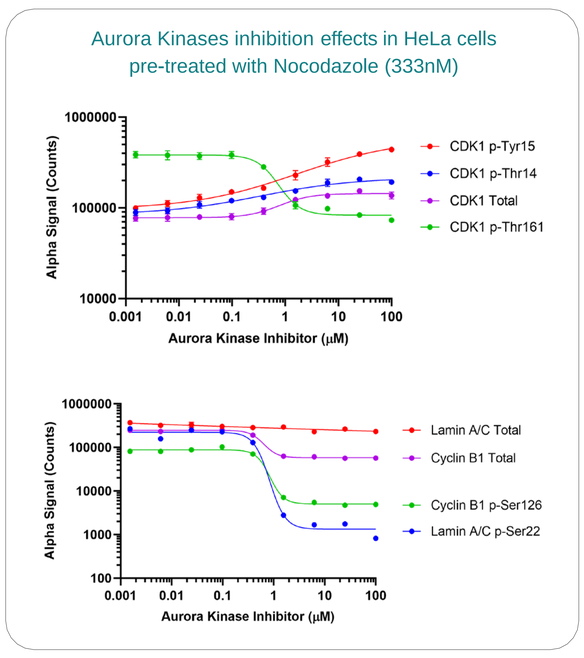
During the late G2 phase at the onset of mitosis, the gradual accumulation of Cyclin B1 promotes the formation of the Cyclin B1-CDK1 complex. This complex is initially inactive due to inhibitory phosphorylation of CDK1 on Tyr15 and Thr14 by the kinases Wee1 and Myt1. The phosphatase CDC25C removes these inhibitory phosphates, thus activating the cyclin B1-CDK1 complex which is phosphorylated on Thr161 and drives the cell into mitosis.
In the presence of nocodazole, a well-known microtubule inhibitor, cells are unable to proceed past metaphase, leading to an accumulation of cells in mitosis with high levels of active cyclin B1-CDK1 as well lamin A/C phosphorylated on Ser22 and Ser392.



Our results indicated that Nocodazole treatment caused a significant activation of CDK1 by triggering phosphorylation of Thr161 and dephosphorylation of the inhibitory residues Thr14 and Tyr15. In addition, higher doses of nocodazole led to an increase of CyclinB1 expression level alongside an increase of its phosphorylation status Cyclin B1 levels. These events resulted in an increase of Lamin A/C phosphorylation at Ser22 with no significant changes in the expression levels.

Inhibition of CDK1 and Cyclin B1 induces downregulation of Lamin A/C phosphorylation.

Aurora kinases are a family of serine/threonine kinases that play a crucial role in mitosis, particularly in regulating chromosome alignment, spindle assembly, and cytokinesis. Aurora kinases inhibition results in significant effects on the cell cycle, particularly on the cyclin B1-CDK1 complex and its downstream events, thereby affecting the overall mitotic entry and progression. Inhibition of Aurora kinase inhibition alters the timing and regulation of lamin A/C phosphorylation, leading to defects in nuclear envelope dynamics.



Our results indicated that Aurora Kinase inhibition resulted in the dephosphorylation of CDK1 on the active Thr161 residue alongside phosphorylation of inhibitory residues Thr14 and Tyr15, together with a modest increase in CDK1 expression level. These events are associated with a significant increase of the expression and phosphorylation of Cyclin B1 (Ser126) as well as phosphorylated Lamin A/C (Ser22), while Lamin A/C expression levels remained unchanged.

5 Material

Supplier	Product Name	Part Numbers
Revvity	Lamin A/C p-S22	ALSU-PLAM-A500
Revvity	Lamin A/C Total	ALSU-TLAM-A500
Revvity	Cyclin B1 p-S126	ALSU-PCYCB1-A500
Revvity	Cyclin B1 Total	ALSU-TCYCB1-A500
Revvity	CDK1 p-T14	ALSU-PCDK1-A500
Revvity	CDK1 p-Y15	ALSU-PCDK1-B500
Revvity	CDK1 p-T161	ALSU-PCDK1-C500
Revvity	CDK1 Total	ALSU-TCDK1-A500
Revvity	96 wells Culture-plate	6005680
Revvity	OptiPlate 384 wells	6007290
Sigma	Nocodazole	M1404
Sigma	Aurora Kinase Inhibitor	189406

6 Summary

AlphaLISA™ is a bead-based technology that provides a fast and reliable method for studying biomolecular interactions in a microplate format. This study showcases the power of the AlphaLISA™ SureFire Ultra platform in measuring changes in the expression and phosphorylation of key cell regulatory proteins.

We observed significant changes in A549 and HeLa cells treated with two compounds with opposite effects: Nocodazole and an Aurora Kinase Inhibitor. The low cell numbers required for each assay highlight the sensitivity of the AlphaLISA™ SureFire Ultra kits. Additionally, the ability to lyse cells and test multiple targets from the same lysate reduces technical errors associated with running parallel cell treatments across multiple assay plates.