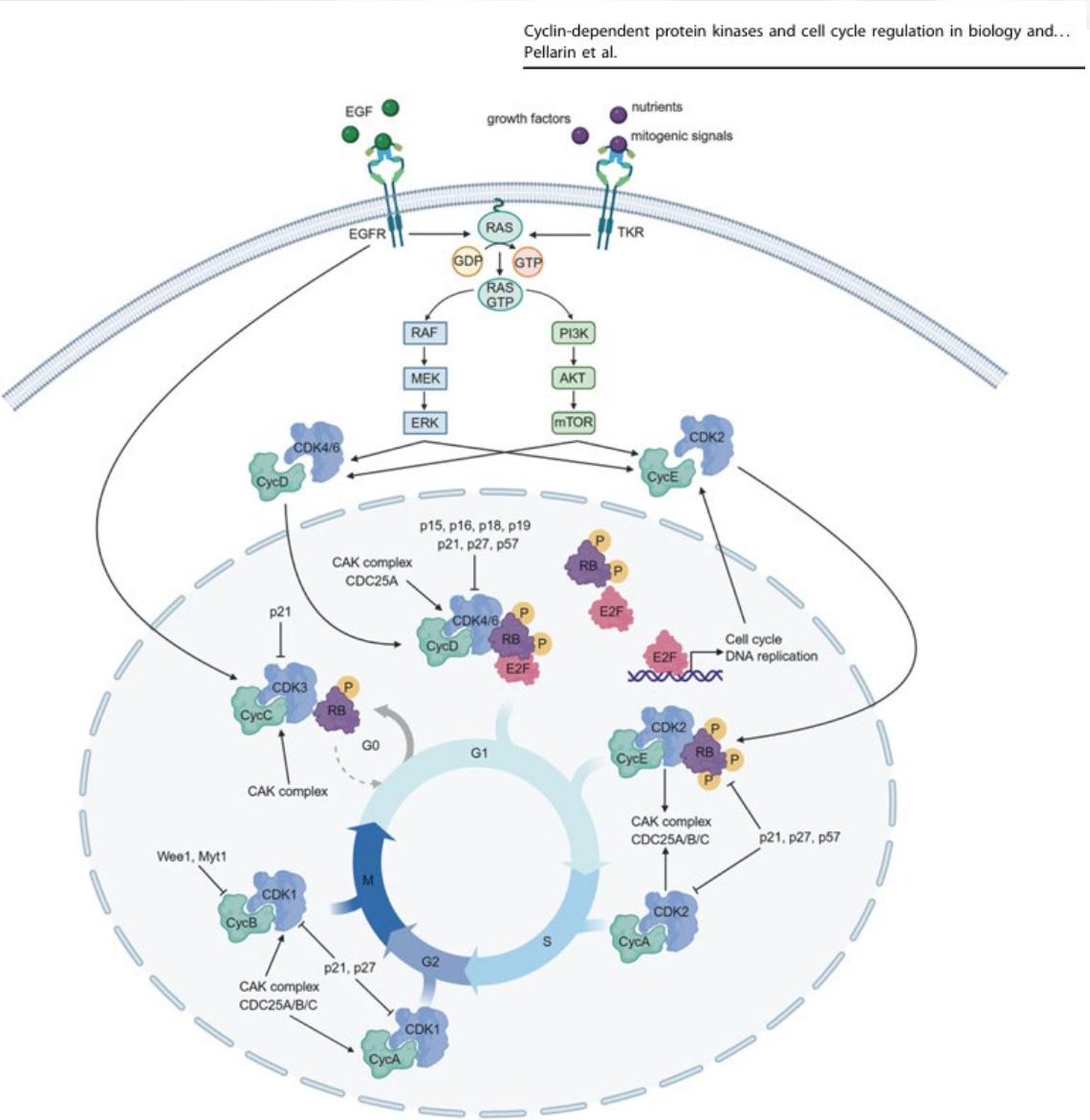
***PhosphoSens*® Cell Lysate Activity Assay Format**

CDK2-CycE1 Assay Validation Using the AQT1170 Selective Sensor Peptide

HGNC Name: CDK2-Cyclin E1
Long Names: Cyclin-dependent kinase 2-Cyclin E1

Cyclin-Dependent Kinases (CDKs) in Cancer



Cell Cycle Progression via EGFR and Receptor Tyrosine Kinase Signaling. EGFR stimulation promotes the activation of the CyclinC-CDK3 complex, enabling the cell to exit quiescence (G0) and enter the G1 phase by priming RB phosphorylation. Activation of various Receptor Tyrosine Kinases (RTKs) can similarly promote G1 progression through signal cascades involving RAS-GTP and its downstream RAF/MEK/ERK and PI3K/AKT/mTOR pathways. These signaling cascades lead to the formation and activation of CyclinD-CDK4/6 complexes and their translocation to the nucleus. In the nucleus, CyclinD-CDK4/6 complexes further phosphorylate RB. The inhibition of RB allows the accumulation of E2F on DNA, promoting the transcription of genes essential for cell cycle progression and DNA replication. Subsequently, the activation of the CyclinE-CDK2 complex drives the transition from G1 to S phase by hyper-phosphorylating RB, enabling cell cycle progression independently of growth factor stimuli (bypassing the restriction point). The accumulation of CyclinA and the displacement of CyclinE from the CyclinE-CDK2 complex facilitate the formation of the CyclinA-CDK2 complex, which drives S phase entry, progression, and DNA synthesis. Following DNA replication, the CyclinA-CDK1 complex triggers entry into mitosis. This is followed by the formation and activation of the CyclinB-CDK1 complex, which is necessary for the completion of proper cell division. The roles of activating proteins (such as CAK and CDC25A/B/C) and inhibitory proteins (such as CDK inhibitors, WEE1, and MYT1) on specific cyclin-CDK complexes are indicated by black arrows. Abbreviations used: CAK complex (CDK Activating Kinase complex) and CDC25 (Cell Division Cycle 25). Adapted from 1. "Cell Cycle Checkpoints", "RAS Pathway", by BioRender.com, 2024. 2. Pellarin, Dall'Acqua, Favero, et al. Cyclin-dependent protein kinases and cell cycle regulation in biology and disease. *Nature: Signal Transduction and Targeted Therapy* **10**:11, 1-62 (2025).

CDK1, CDK2, CDK4, and CDK6 are all high priority targets for lysate assays.

Regulation of Cyclin Levels During the Cell Cycle Control CDK Activity

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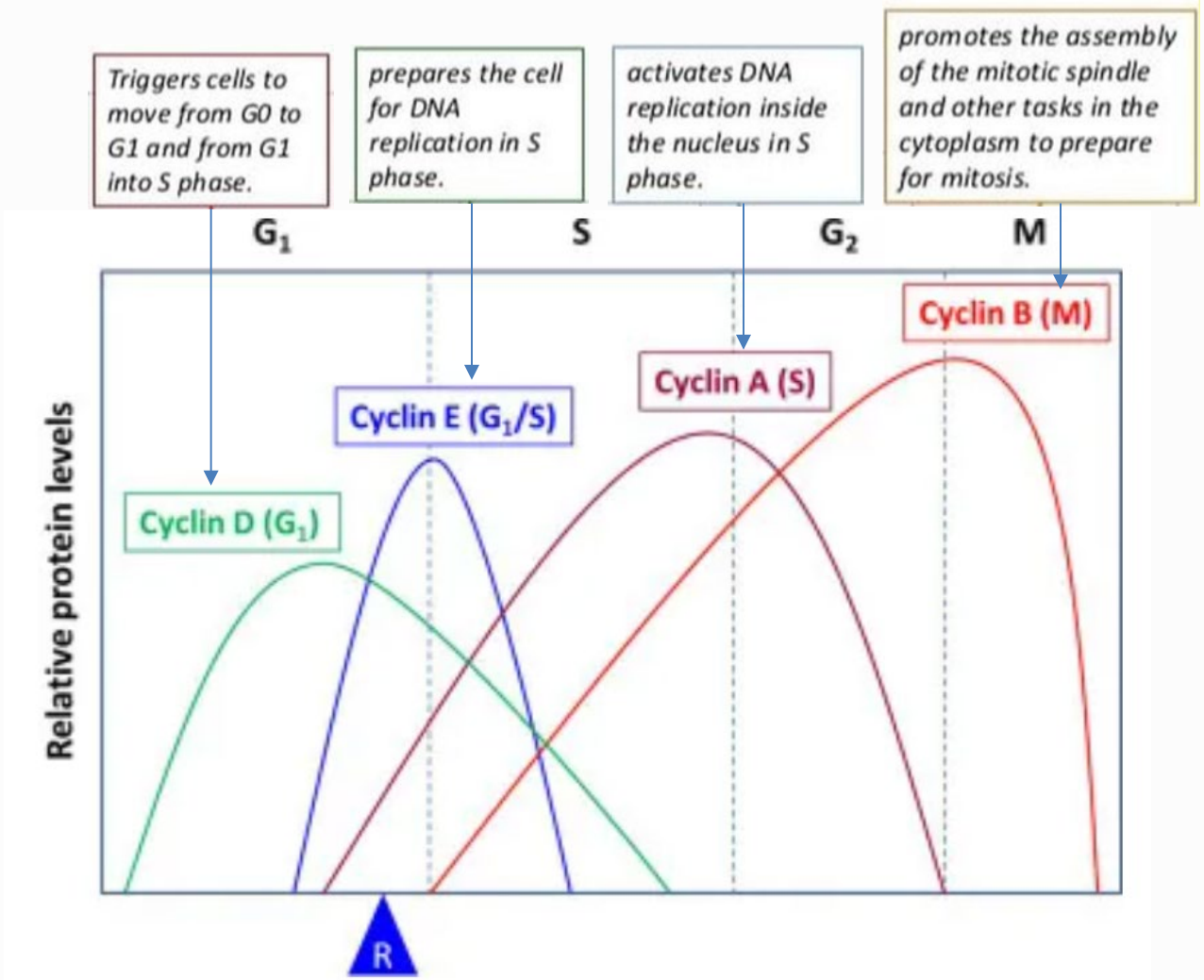
Thymidine is a DNA synthesis inhibitor that arrests cells at the G1/S boundary, prior to DNA replication.



The cell cycle is driven by cyclin-cyclin-dependent kinase (CDK) complexes, which are regulated by the relative protein levels of the four cyclins, since they undergo a cycle of synthesis and degradation in each phase of the cell cycle. The G1/S checkpoint (also called the G1 checkpoint, restriction or R point) occurs near the end of the G1 phase, just before the entry into S phase. In mammalian cells, this is a point at which cells typically arrest the cell cycle if environmental conditions are unfavorable for cell division, such as the presence of DNA damage or a lack of growth factors. The G1 checkpoint is controlled by the inhibition of CDK4 & 6 by INK4 and CDK2 by Cip/Kip families of CDK Inhibitors. Degradation of INK4 and activation of CDK4/6-Cyclin D complexes synthesis of new proteins, including cyclin E. The rise in cyclin E (a G 1 /S cyclin) levels and the activity of CDK2/CyclinE drive the cell past a restriction point, with irreversible commitment to advancing to DNA synthesis. **Therefore, CDK2/CyclinE activity in crude lysates is a critical measure to understand CDK regulation at the G1/S transition.**

Adapted from: https://abdominalkey.com/the-cell-cycle/

High Selectivity for CDK2-CycE1 with the AQT1170 Sensor Peptide

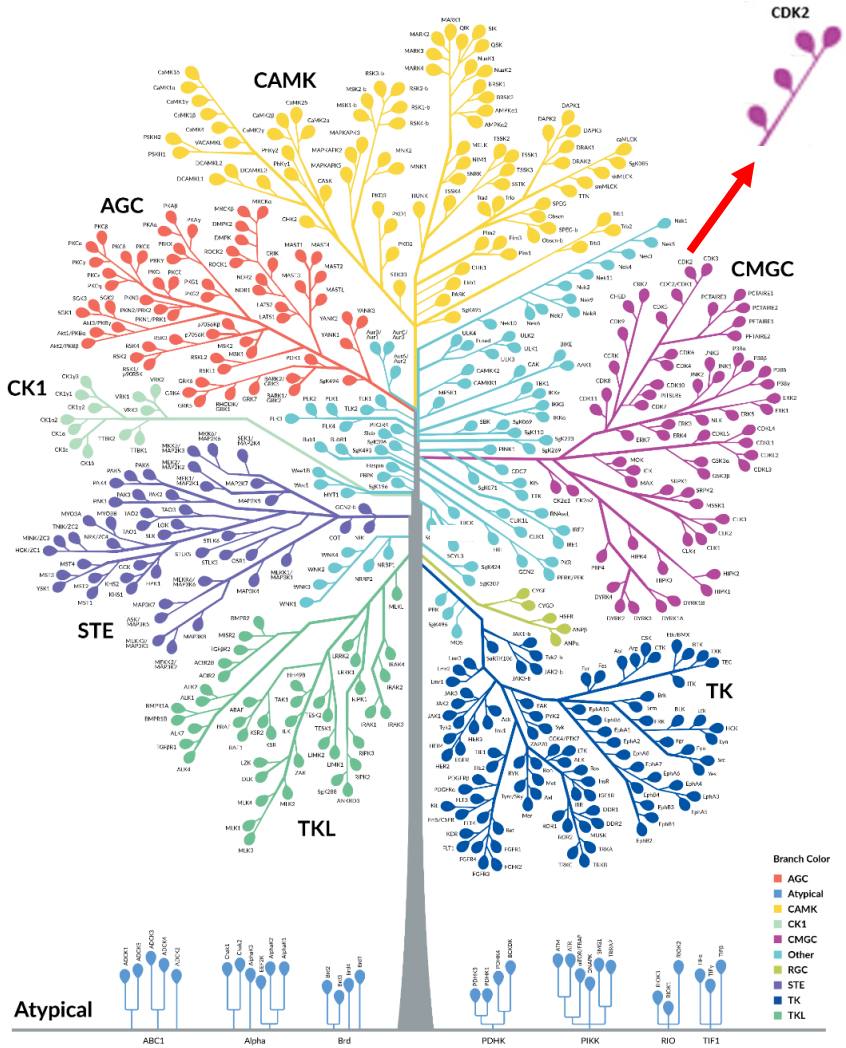
Top Hits with AQT1170 in Kinome Profiling with 36 Wild-type Kinases

Kinase target	Enzyme con	Average (RFU/pmol/min)	Selectivity Ratio	% Activity
CDK2/CycE1	0.50	8696	1.0	100
CDK3/CycE2	0.44	1323	6.6	15
CDK1/CycE1	1.0	433	20	5.0
CDK1/CycA1	1.1	222	39	2.6
CDK3/CycE1	3.0	194	45	2.2
CDK5/p25	2.0	125	69	1.4
CDK2/CycA1	0.30	70	125	0.80
CDK2/CycA2	2.0	57	153	0.66
CDK2/CycE2	1.8	56	155	0.65
p38g	0.40	34	256	0.39
CDK1/CycA2	5.0	30	286	0.35
CDK5/p35	3.0	19	467	0.21
CDK4/CycD1	27	9.4	925	0.11
CDK1/CycB1	3.0	6.7	1306	0.077
CDK9/CycT1	15	3.6	2398	0.042
CDK15/CycB1	4.0	2.9	2983	0.034
CDK4/CycD2	2.7	2.4	3665	0.027
CDK4/CycD3	20	2.0	4292	0.023
CDK6/CycD3	5.4	0.16	55245	0
CDK9/CycK	15	-0.46	-19098	-0.005
CDK11(19)/CycC	40	-0.73	-11868	-0.008
CDK16/CycY	20	-0.91	-9582	-0.010
CDK7/CycH/MAT1	10	-1.1	-7825	-0.013
BTN-CDK8/CycC	40	-1.2	-7536	-0.013
CDK12/CycK	20	-1.3	-6859	-0.015
CDK18/CycY	5.0	-1.3	-6815	-0.015
CDK17/p35	46	-1.3	-6562	-0.015
CDK6/CycD2	7.6	-2.5	-3548	-0.028
CDK13/CycK	15	-2.6	-3394	-0.029
BTN-CDK10/CycQ	40	-2.8	-3132	-0.032
CHK1	5.0	-3.9	-2213	-0.045
CDK6/CycD1	3.0	-4.3	-2032	-0.049
CDK17/CycY	8.9	-4.9	-1789	-0.056
CDK2/CycO	1.4	-6.6	-1316	-0.076
CDK7/CycH1	7.0	-9.5	-912	-0.11
CDK9/CycT2	1.8	-10	-869	-0.12

The kinase with the highest Reaction Rate (RFU/pmole/min) in this kinome profiling with AQT1170 was CDK2-CycE1, with a reaction rate of 8,696 RFU/pmole/min.

The kinase with the next-highest activity, CDK3-CycE2, had 15% the activity of CDK2-CycE1. However, at the G1/S boundary of the cell cycle, CDK2/Cyclin E1 activity is high, while CDK3 activity is low; thus, AQT1170 shows high selectivity for CDK2/E1 in lysates from cells synchronized to G1/S. The remaining kinases showed signals at least 20-fold lower than those of CDK2-CycE1. As cells progress from the G1/S boundary into and through S-phase, CDK2/Cyclin A activity will rise, but AQT1170 shows a signal of <1% with these complexes.

As in this report, one can also use lysates from cell lines shown to be CDK2-dependent via knockout studies, such as NIH-OVCAR3 Cells (Zhang et al., 2022, Cancer Research 82:11, 2171-2184).



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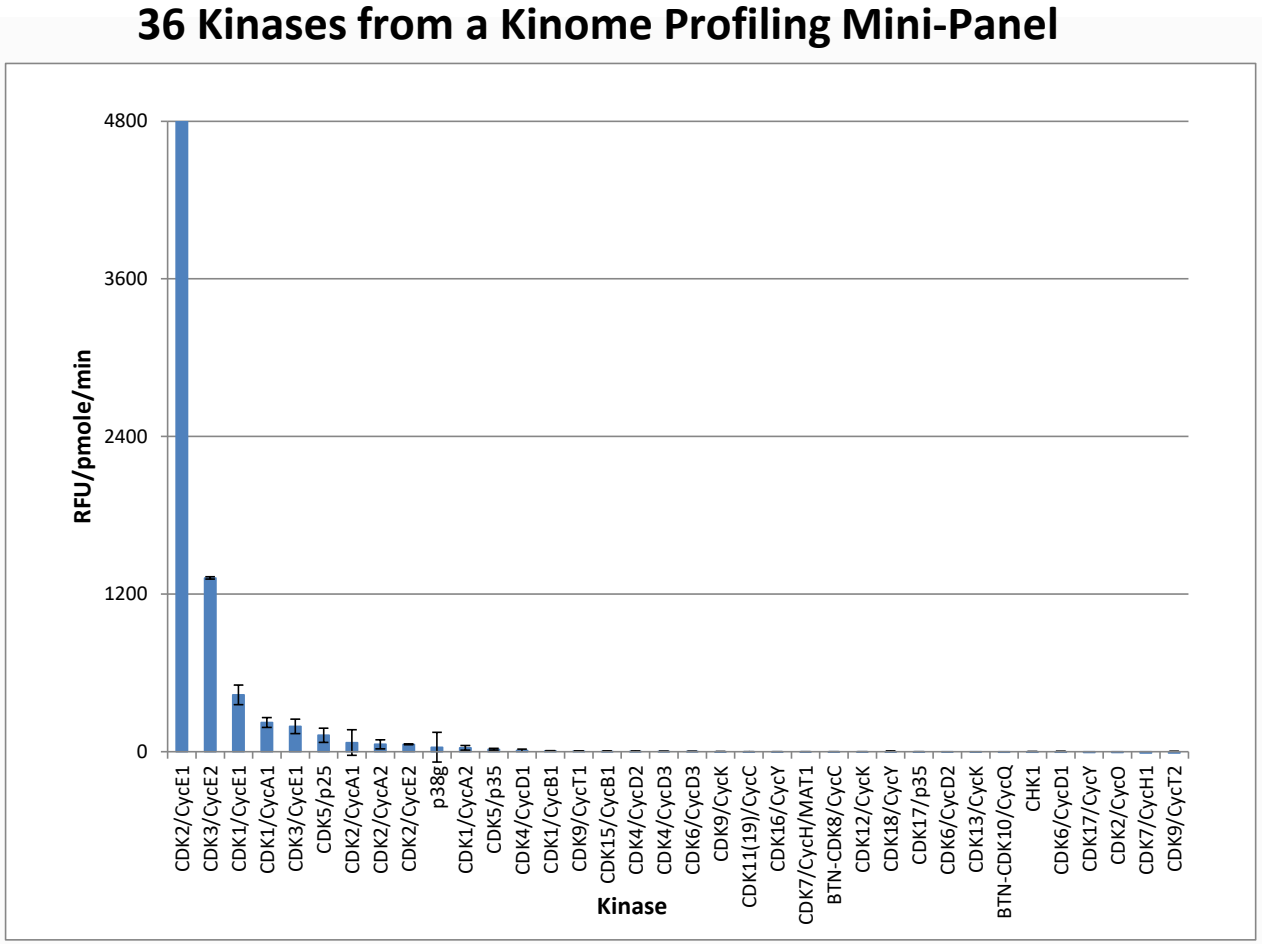
Selectivity of AQT1170 with the 36 Kinases from AQT's Kinome Profiling Per Slide 5



High-throughput Milestone Optimization led to the identification of the AQT1170 sensor peptide substrate.

AQT1170 shows selectivity for CDK2-CycE1, a critical requirement for accurately measuring the activity of CDK2-CycE1 in crude lysates

Outline for PhosphoSens-Lysate Assay Validation

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Lysate Source:

- NIH-OVCAR3 (ATCC, HTB-161) cell line untreated or treated with thymidine (Sigma-Aldrich, T1895-1G) to synchronize the cells to S Growth Phase (see slide 8 for further details)

Reference Compound Information:

- CDK2-IN-23 (MedChemExpress, HY-162255)

Experimental Validation at AssayQuant:

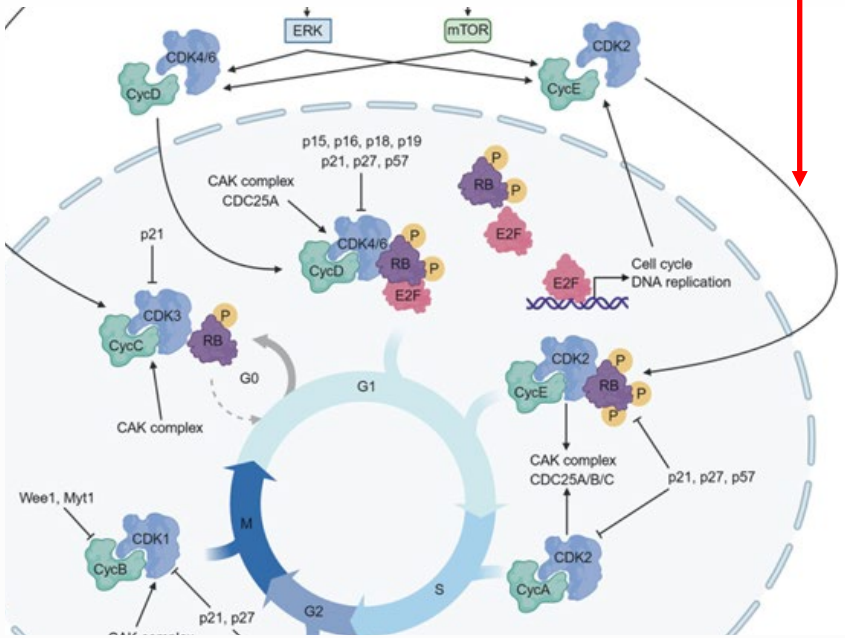
- Cell lysate titrations
- AQT1170 substrate K_m determination
- DMSO Tolerance Test
- Reference Compound IC_{50} Determination
- CDK2-CycE1 (AQT-C29-18G) to serve as positive recombinant protein control
- Phosphopeptide control (AQT1171)
- Assessment of CDK Activity in a CDK2 Dependent Cell Line +/- S Phase Synchronization

CDK2-CycE1 Cell Cycle Regulation

Following the formation and activation of CDK4/6 complexes, further phosphorylation and inactivation of RB, and gene transcription are essential for cell cycle progression and DNA replication. **CDK2-CycE1** is activated which drives the transition from G1 Phase to S Phase by hyper-phosphorylating RB and enabling cell cycle progression independent of growth factor stimuli. CDK2-CycA formation from CDK2 with displaced CycE drives S Phase entry and progression. Blocking cells at the G1/S boundary will elevate CDK2-CycE levels. Pellarin, Dall'Acqua, Favero, *et al.* Cyclin-dependent protein kinases and cell cycle regulation in biology and disease. *Nature: Signal Transduction and Targeted Therapy* **10**:11, 1-62 (2025).

NIH-OVCAR3 Cells were shown to be CDK2 dependent via knockout studies. Zhang, Golomb, and Meyerson. Functional genomic analysis of CDK4 and CDK6 gene dependency across human cancer cell lines. *Cancer Research* **82**:11, 2171-2184 (2022) and CDK2-IN-23 was shown to have CDK2 specificity and to inhibit recombinant CDK2-CycE1 in AQT internal studies. Studies for CDK2-CycE1 with AQT1170 were performed with NIH-OCAR3 Cells synchronized to S Phase to increase CDK2 levels with CDK2-IN-23 used as an inhibitor control.

Cell Cycle Regulation of G1/S Restriction by CDK2-CycE1



Preparation of Crude Cell Lysates from NIH-OVCAR3 ± Cell Cycle Synchronization at G1/S

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Thymidine is a DNA synthesis inhibitor that arrests cells at the G1/S boundary, prior to DNA replication. Here, we used a double thymidine block protocol to synchronize cells at G1/S boundary.

1. NIH-OVCAR3 (passage 3-5) were plated in T-75 flasks and grown to 75% confluency over 48 hours at 37°C in RPMI-1640 Medium (ATCC, 30-2001) with 10% FBS (ATCC, 30-2020) and 1% PenStrep (30-2300) in an atmosphere of 5% CO_2. NIH-OVCAR3 cells were incubated with 2.5 mM Thymidine for 24 hours (**first block**).

2. After 24 hours, NIH-OVCAR3 medium was removed and replaced with fresh media for 4 hours (first release) and then incubated a second time with 2.5 mM Thymidine for 24 hours (**second block**). After these 48 hours, the media was removed from the NIH-OVCAR3 cells. The cells were then washed once with PBS with calcium and magnesium salts, scraped into 6 mL of this buffer, and then centrifuged for 5 minutes at 2,500 x g. Liquid was removed, and the pellets were placed on ice and immediately lysed with 100-300 µL of cold Cell Extraction Buffer (CEB) containing protease but not phosphatase inhibitors, gently triturated to ensure solubilization, and then centrifuged for 5 minutes at 12,000 x g to pellet the cellular debris. Lysate supernatants were removed, aliquoted, supplemented with 10% glycerol, and used immediately or frozen at –80 °C; each aliquot was **used only once (one freeze/thaw cycle)**. Long-term storage of lysates is not recommended, *as activity may decline over time.*

Note: NIH-OVCAR3 cells were treated with thymidine to synchronize them to S Phase and increase CDK2-CycE1 levels

Cell Extraction Buffer (CEB) with protease inhibitors:

- 50 mM HEPES, pH 7.4
- 150 mM NaCl
- 2 mM EGTA
- 1 mM DTT
- 1% Triton X-100
- Protease Inhibitor Cocktail diluted 60-fold into lysis buffer

Note: No phosphatase inhibitors are added to the CEB-B because there are phosphatases that are needed to activate CDKs.

NIH-OVCAR3 Lysate Titration with the AQT1170 Sensor Peptide

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5

1 mM ATP

1.2 mM DTT

0.012% Brij-35

1% glycerol

0.2 mg/ml BSA

0.54 mM EGTA

10 mM $MgCl_2$

15 µM AQT1170 sensor peptide substrate

0, 0.010, 0.020, 0.039, 0.078, 0.16, 0.31, 0.63, 1.3, 2.5, 5.0, 10 µg/well **crude cell lysate** from NIH-OVCAR3 cells synchronized to G1/S Phase of cell growth

Reaction Set Up:

20 µL Reaction Mix with AQT1170, ATP, & DTT
Seal plate and incubate at 30 °C for 15 minutes to equilibrate
5 µL Enzyme dilution buffer (EDB) with CEB Lysate Buffer (1x) or NIH-OVCAR3 Lysate (5x in EDB)
25 µL Final reaction volume

Reaction was run at 30˚C for 480 minutes in Corning, low volume 384-well, white flat-bottom polystyrene NBS microplates (Cat. #3824) at 25 µL final well volume after sealing using optically-clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

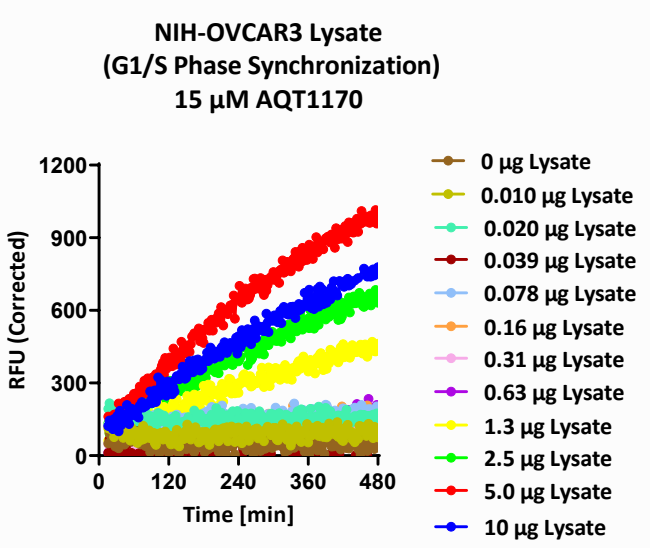
Notes:

Enzyme Dilution Buffer (EDB): 20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

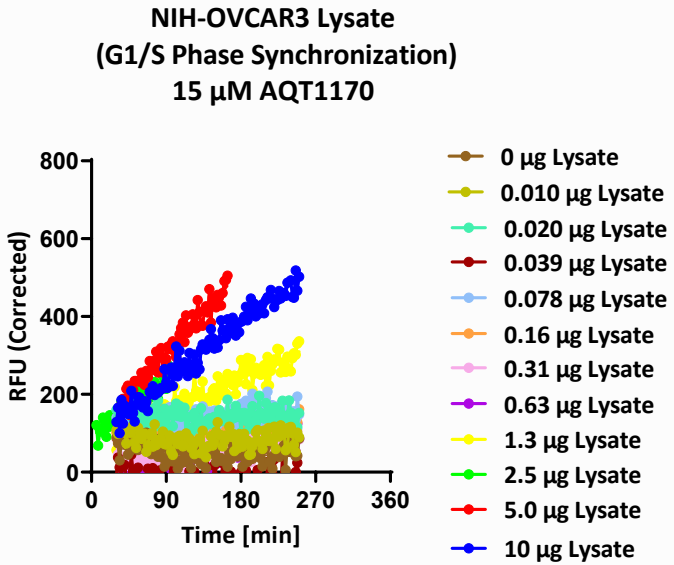
Lysate Titration with the AQT1170 Sensor Peptide

Progress curves and determination of linearity

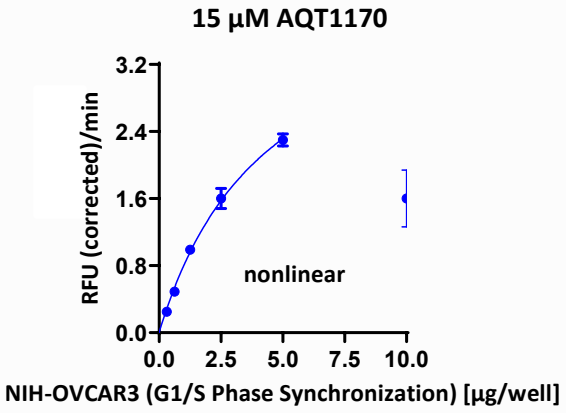
Complete Progress Curves



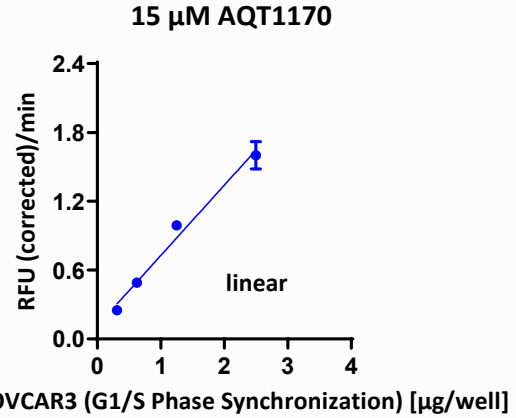
Linear Region of Progress Curves



Nonlinear Reaction



Linear Reaction



The CDK2 Lysate Assay is linear from 0.63 – 2.5 µg/well of lysate (4-fold)

CDK2-CycE1 Sensor Peptide K_m Determination

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5

1 mM ATP

1.2 mM DTT

0.012% Brij-35

1% glycerol

0.2 mg/ml BSA

0.54 mM EGTA

10 mM $MgCl_2$

0, 0.20, 0.39, 0.78, 1.6, 3.1, 6.3, 13, 25, 50, and 100 µM **AQT1170 sensor peptide substrate**

2.0 µg/well NIH-OVCAR3 crude cell lysate from NIH-OVCAR3 cells synchronized to G1/S Phase of cell growth

Reaction Set Up:

2.5 µl 10X AQT1170 Substrate dilutions
17.5 µL Reaction Mix with ATP & DTT
Seal plate and incubate at 30 °C for 15 minutes to equilibrate
5 µL Enzyme dilution buffer (EDB) with CEB Lysate Buffer (1x) or NIH-OVCAR3 Lysate (5x in EDB)
25 µL Final reaction volume

Reaction was run at 30˚C for 480 minutes in Corning, low volume 384-well, white flat-bottom polystyrene NBS microplates (Cat. #3824) at 25 µL final well volume after sealing using optically-clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

Notes:

Enzyme Dilution Buffer (EDB):20 mM HEPES, pH 7.5, 0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

Sensor Peptide K_m Determination for AQT1170

Titration Curves and K_m Plot and Table

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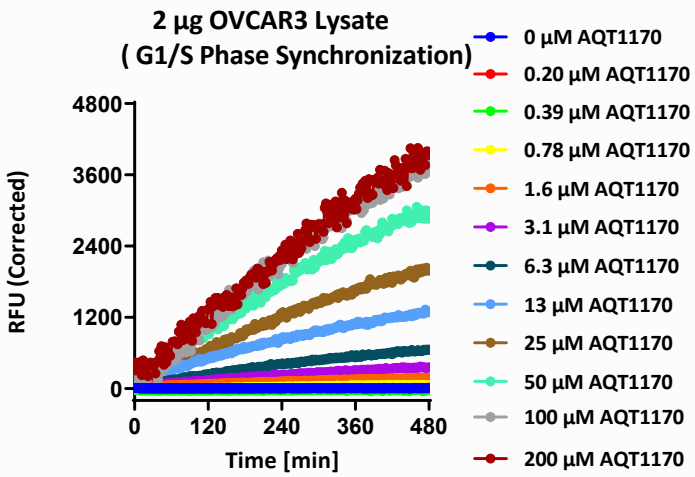
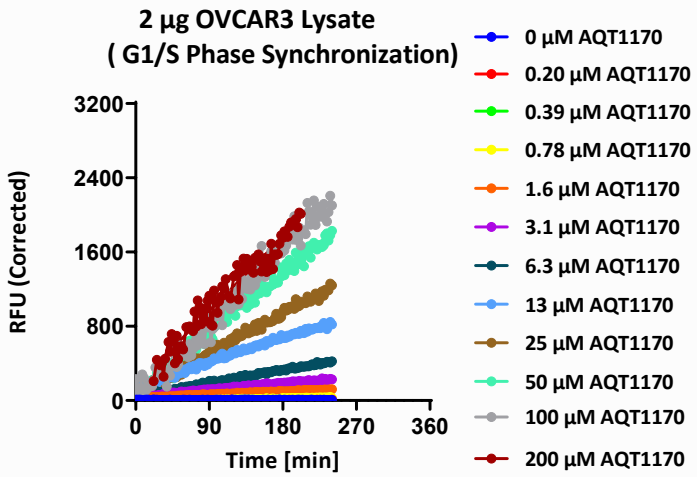
Sensor Peptide Titration Progress Curves

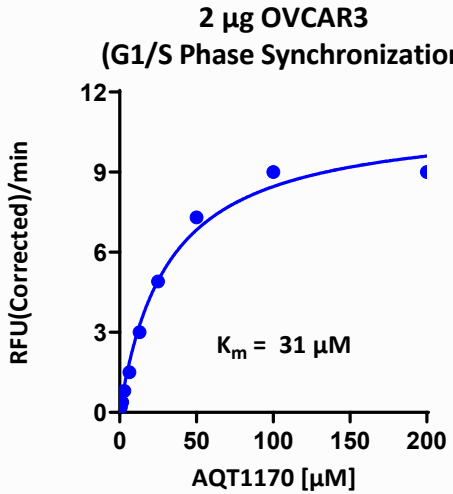
Complete Time Course



Linear Region



Sensor Peptide K_m Plot



Michaelis-Menten	
Best-fit values	
Vmax	11.10
Km	31.27
95% CI (profile likelihood)	
Vmax	9.962 to 12.43
Km	22.77 to 43.05
Goodness of Fit	
Degrees of Freedom	7
R squared	0.9892
Sum of Squares	1.148
Sy.x	0.4050
Constraints	
Km	Km > 0
Number of points	
# of X values	9
# Y values analyzed	9

The K_m value for AQT1170 is 31 µM. We recommend using at 15 µM final concentration.

DMSO Tolerance Test with the AQT1170 Sensor Peptide

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5

1 mM ATP

1.2 mM DTT

0.012% Brij-35

1% glycerol

0.2 mg/ml BSA

0.54 mM EGTA

10 mM $MgCl_2$

15 µM AQT1170 sensor peptide substrate

2.0 µg/well NIH-OVCAR3 crude cell lysate from NIH-OVCAR3 cells synchronized to G1/S Phase of cell growth

0-10% DMSO

Reaction Set Up:

2.5 µL 10X DMSO Titration
17.5 µL Reaction Mix with AQT1170, ATP & DTT
Seal plate and incubate at 30 °C for 15 minutes to equilibrate
5 µL Enzyme dilution buffer (EDB) with CEB Lysate Buffer (1x) or NIH-OVCAR3 Lysate (5x in EDB) 25 µL Final reaction volume

Reaction was run at 30˚C for 480 minutes in Corning, low volume 384-well, white flat-bottom polystyrene NBS microplates (Cat. #3824) at 25 µL final well volume after sealing using optically-clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

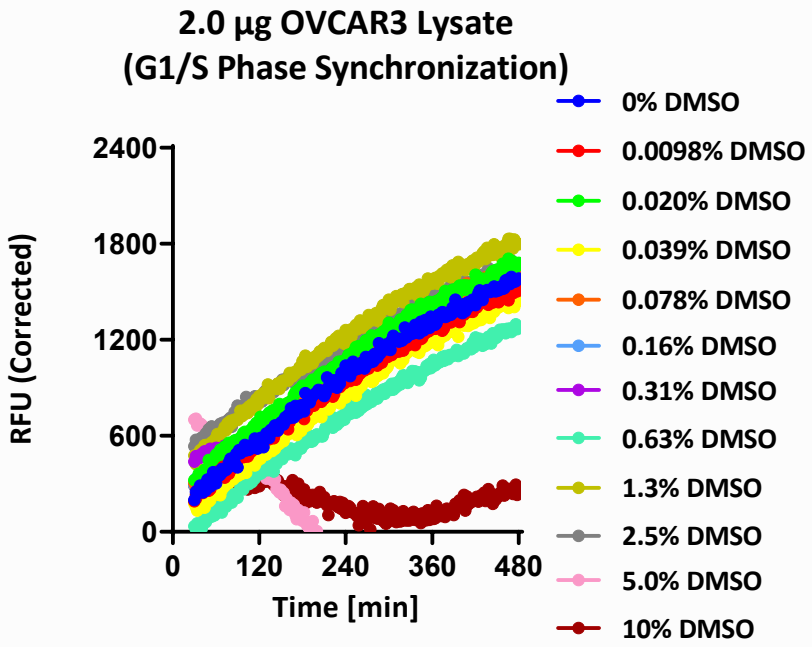
Notes:

Enzyme Dilution Buffer (EDB):20 mM HEPES, pH 7.5,0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

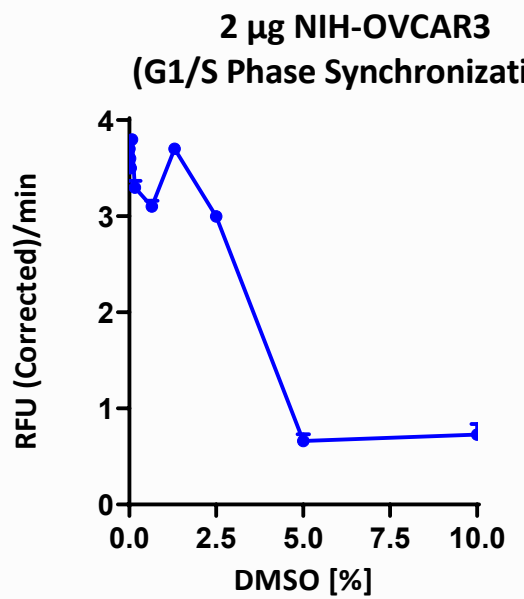
DMSO Tolerance Test with the AQT1170 Sensor Peptide

Titration Curves and Inhibition Plot

Complete Progress Curves



Reaction Rate vs [DMSO] Plot



No significant loss in enzyme activity was observed up to 2.5% DMSO. A complete loss in signal was observed at both 5.0% and 10% DMSO final.

IC_{50} Determination with the AQT1170 Sensor Peptide

Reaction Conditions and Set Up

Reaction Conditions:

54 mM HEPES, pH 7.5
1.0 mM ATP
1.2 mM DTT
0.012% Brij-35
1% glycerol
0.2 mg/ml BSA
0.54 mM EGTA
10 mM $MgCl_2$
15 µM AQT1170 sensor peptide substrate

Compounds:

- CDK2-IN-23 was titrated with 3-fold dilutions in 100% DMSO at 50X the final concentrations and then diluted 50-fold into the assay for final concentrations from 0-10 µM in 2% DMSO.

Cell Lysate for IC_{50} determination:

2.0 µg/well NIH-OVCAR3 crude cell lysate from NIH-OVCAR3 cells synchronized to G1/S Phase of cell growth

Reaction Set Up:

0.5 µL 50X CDK2-IN-23 diluted in 100% DMSO or DMSO alone
19.5 µL Reaction Mix with AQT1170, ATP & DTT
Seal plate and incubate at 30 °C for 15 minutes to equilibrate
5 µL Enzyme dilution buffer (EDB) with CEB Lysate Buffer (1x) or NIH-OVCAR3 Lysate (5x in EDB) 25 µL Final reaction volume

Reaction was run at 30˚C for 480 minutes in Corning, low volume 384-well, white flat-bottom polystyrene NBS microplates (Cat. #3824) at 25 µL final well volume after sealing using optically-clear adhesive film (TopSealA-Plus plate seal, PerkinElmer [Cat. #6050185]) in a Biotek Synergy Neo 2 microplate reader with excitation (360 nm) and emission (485 nm) wavelengths.

Notes:

1X Enzyme Dilution Buffer (EDB) is used to dilute enzyme and for the blank. Composition is 20 mM HEPES, pH 7.5,0.01% Brij-35, 5% Glycerol, 0.5 mM EGTA, 1 mM DTT, 1 mg/ml Bovine Serum Albumin.

IC_{50} Determination of CDK2-IN-23 Using the AQT1170 Sensor Peptide with Lysates from NIH-OVCAR3 Cells Synchronized at G1/S

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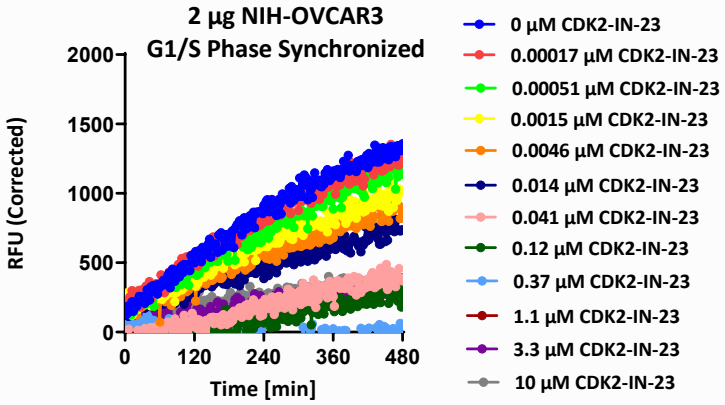
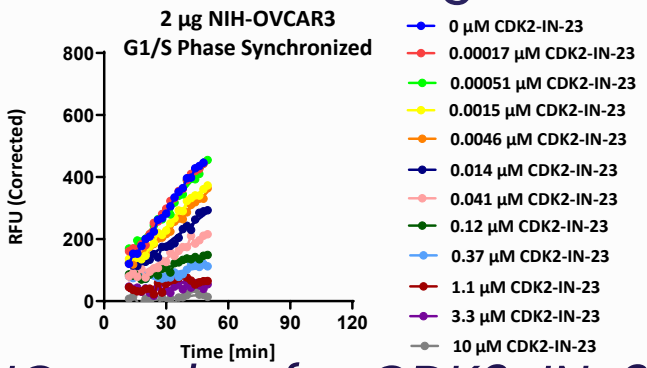
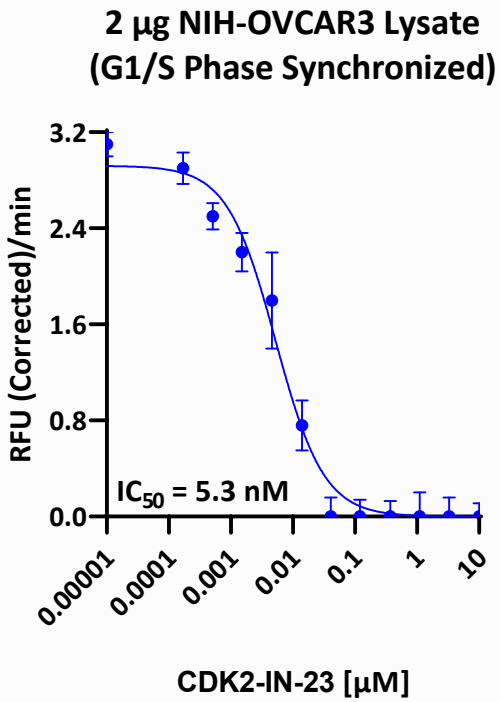
Full Progress Curves



Progress Curves for Linear Region



IC_{50} Curve



[Inhibitor] vs. response -- Variable slope (four parameters)	
Best-fit values	
Bottom	= 0.000
Top	2.921
IC50	0.005278
HillSlope	-1.117
logIC50	-2.278
Span	2.921
95% CI (profile likelihood)	
Top	2.642 to 3.259
IC50	0.003188 to 0.008081
HillSlope	-1.769 to -0.7785
logIC50	-2.497 to -2.093
Goodness of Fit	
Degrees of Freedom	9
R squared	0.9870
Sum of Squares	0.2374
Sy.x	0.1624
Constraints	
Bottom	Bottom = 0
IC50	IC50 > 0
Number of points	
# of X values	12
# Y values analyzed	12

IC_{50} value for CDK2-IN-23 in NIH-OVCAR3 Cell Lysate from cells synchronized at G1/S *is 5.3 nM.*

CDK2-CycE1 Lysate Activity Assay Using the AQT1170 Sensor Peptide

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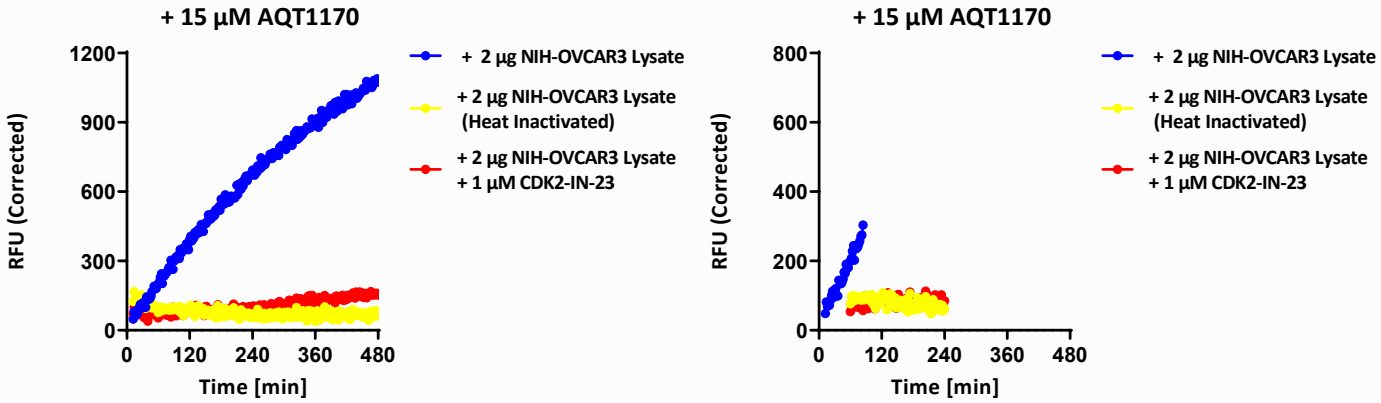
1) Full Time Course (0-480 min.) & Linear Range (0-240 min.) with 2.0 µg/well

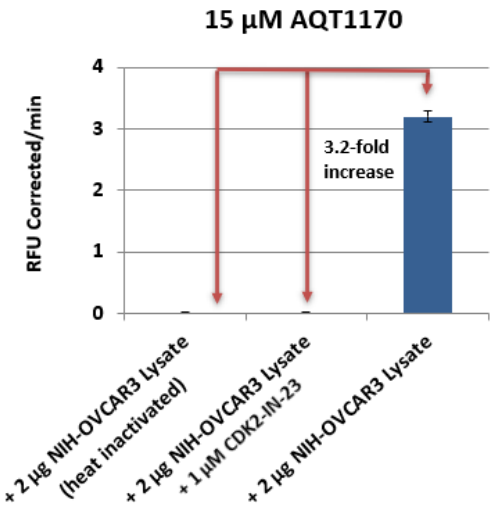


2) Reaction Rates to Assess Change

	Reaction Rate (RFU/min)	Change
2.0 µg NIH-OVCAR3 lysate (no treatment)	3.2 ± 0.097	
2.0 µg NIH-OVCAR3 lysate, Heat Inactivated	0 ± 0.024	100% inhibition CDK2 Activity
2.0 µg NIH-OVCAR3 lysate, + 1.0 µM CDK2-IN-23	0 ± 0.022	100% inhibition CDK2 Activity

Crude lysate samples: The AQT1170 sensor peptide (15 µM) was used to generate **1)** Full progress curve time course and the linear range. The reaction rates (RFU Corrected values [Total – Background]/min. +/- standard deviations) are the slope of the linear region of each progress curve, which are presented in **2)**, highlighting complete elimination of signal with heat inactivation and by CDK2-IN-23, a CDK2 inhibitor.

Note: The amount of activation depends on several factors, including cell type and passage number, percentage of cells synchronized, and the nature, concentration, and duration of the exposure to pathway stimuli or inhibitors. The total amount of CDK2-CycE1 protein can be determined by Western Blotting or an ELISA; however, with the short stimulation times typically used, these levels are not expected to change.



CDK2-CycE1 Recombinant Activity Assay with AQT1170 Sensor Peptide and Phosphocontrol AQT1171

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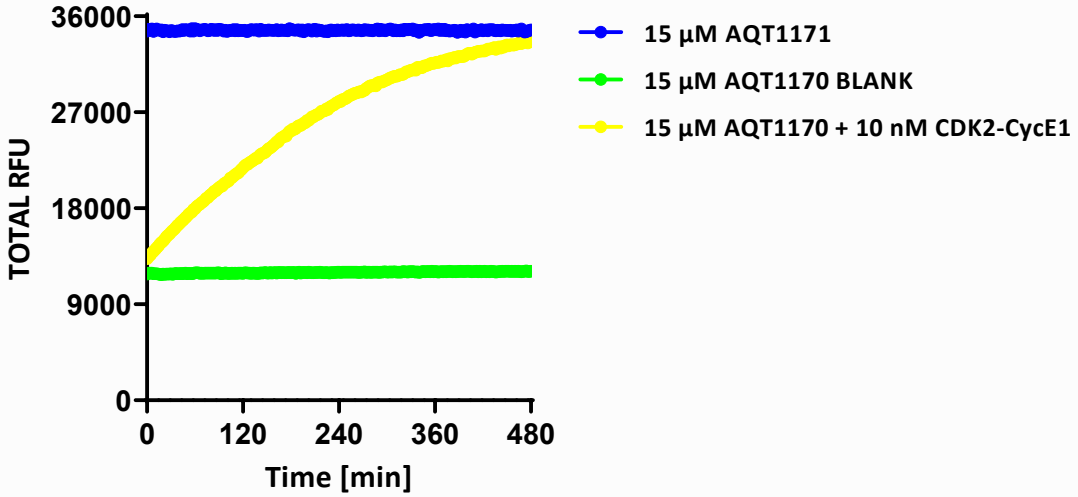
Full Time Course (0-8 hours)

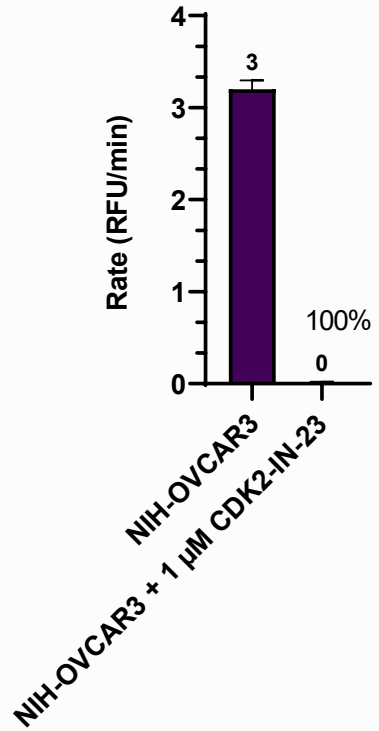


Time course for the phosphorylation of AQT1170 sensor peptide substrate (15 µM) ± 10 nM CDK2-CycE1 recombinant enzyme, compared to the signal with the AQT1171 phosphopeptide control (15 µM): Full-length CDK2-CyclinE1 protein fully phosphorylated the AQT1170 sensor peptide substrate by 8 hours, as shown by convergence with the signal obtained with the AQT1171 phosphopeptide positive control (a flat horizontal line defining the maximum RFU with this sensor peptide). The signal with AQT1171 is used to convert RFU (Corrected) values to nmoles of phosphopeptide product/minute.

AQT1171 is a chemically synthesized phosphopeptide control for AQT1170

CDK2-CycE1 Lysate Activity Assay Using AQT1170 Sensor Peptide with a CDK2-dependent Cell Line (NIH-OVCAR3) with Synchronization at G1/S of the Cell Cycle

2 µg Lysate/well with AQT1170 Sensor Peptide



NIH-OVCAR3 cells (CDK2 dependent) were treated as described on page 9 to optimize levels of CDK2-CycE1 by cell cycle synchronization at the G1/S boundary using a double-thymidine block, and lysates were prepared for activity assays with the AQT1170 selective sensor peptide for CDK2/Cyclin E. Lysates from these cells were inhibited by 1 µM CDK2-IN-23, a CDK2 selective inhibitor.

Demonstrates CDK2-CycE1 activity in a CDK2 dependent cell line which is inhibited by a CDK2 selective inhibitor

Summary

- The PhosphoSens-Lysate Assay for CDK2-CycE1 using the AQT1170 selective sensor peptide demonstrates a robust, sensitive, and physiologically relevant assay that selectively measures endogenous CDK2-CycE1 activity with all the cellular components and signaling complexes using only 2 µg of crude cell lysate per test.
- Results include:
  - CDK2 activity was observed in NIH-OVCAR3 cell lysate from cells synchronized at G1/S in the cell cycle.
  - NIH-OVCAR3 lysate activity was inhibited by 100% by 1 µM of CDK2-IN-23, a potent, specific CDK2 inhibitor.
  - An IC_{50} value of 5.3 nM was observed for CDK2-IN-23 with NIH-OVCAR3 Lysate (CDK2 dependent).
  - The AQT1170 K_m value for NIH-OVCAR3 Lysate was 31 µM.
  - The CDK2-CycE1 Lysate Assay was sensitive to DMSO, tolerating DMSO up to only 2.5% final concentration.

These CDK activity measurements are direct, highly quantitative, and an easy-to-use assay format. This allows functional assessment of native CDK2-CycE1 activity in complex samples using crude cell lysates or tissue homogenates, thereby providing a more physiological and economical approach to study CDK2-CycE1 kinases.