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## Datasheet for ABIN1380163 Cyclin D1 ELISA Kit



Overview

Quantity:	2 x 96 tests
Target:	Cyclin D1 (CCND1)
Binding Specificity:	pThr286
Reactivity:	Human, Mouse, Rat
Method Type:	Cell ELISA
Application:	ELISA

### Product Details

Sample Type:	Cell Culture Cells
Analytical Method:	Qualitative
Detection Method:	Colorimetric
Specificity:	The Anti-Cyclin D1 (Phospho-Thr286) antibody is a rabbit polyclonal antibody. It was tested on
	Western Blots for specificity. A single protein band was detected. This protein band can be
	blocked by the synthesized immunogen peptide.
	The Anti-Cyclin D1 (Phospho-Thr286) antibody is highly specific for the phospho-peptide in
	comparison to the non-phospho peptide counterpart, through an ELISA.
	The Anti-Cyclin D1 antibody is a rabbit polyclonal antibody. It was tested on Western Blots for
	specificity. A single protein band was detected. This protein band can be blocked by the
	synthesized immunogen peptide.
	The Anti-GAPDH antibody is a mouse monoclonal antibody. It was tested on Western Blots with
	the tissue lysates from human, mouse, and rat for specificity. A single protein band was
	detected from all three lysates.

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#### Characteristics:

1) Cell Line: The cell line must express the target protein. This protocol can be used directly for adherent cells. For suspension cells and loosely attached cells, two steps are required: Coat the plates with 100  $\mu$ L of 10  $\mu$ g/mL Poly-L-Lysine to each well of the 96-well plate for 30 minutes at 37°C before proceeding to Step 1 of Assay Protocol. Use 8% formaldehyde to fix the cells on Step 5 of Assay Protocol.

2) Cell Number and Sensitivity: The number of cells plated onto the 96-well plates depends on the expression level of Cyclin D1 protein in the cells, cell size, treatment conditions and incubation time. The cells used for testing should be around 75-90% confluent. Typically for HeLa cells, seed 30,000 cells per well overnight for treatment the following day. The Cyclin D1 (Phospho-Thr286) Colorimetric Cell- Based ELISA Kit can detect Phospho-Cyclin D1 expression in as little as 5,000 HeLa cells.

3) Cell Treatment: The cells can be treated with inhibitors, activators, stimulators (ie. chemicals, proteins/peptides) or a combination of the substances listed above. The cells can be treated with UV and serum starvation to meet the needs of the end-user.

4) Positive and Negative Controls: Mouse Anti-GAPDH antibody (included) should be used to detect the internal positive controls for normalization of OD values of the target protein. The negative controls are HRP-Conjugated Anti-Rabbit IgG antibody and HRP- Conjugated Anti-Mouse IgG antibody alone in different wells (without the primary antibodies). Both positive and negative controls should be performed in the same plate with the Phospho-Cyclin D1 target experiments.

5) Accuracy and Precision: Each condition should be performed in duplicate or in triplicate.

Components:	96-Well Cell Culture Clear-Bottom Microplate: 2 Plates
	TBS: 24 mL (10x), Clear
	Quenching Buffer: 24 mL (1x), Clear
	Blocking Buffer: 50 mL (1x), Clear
	Wash Buffer: 50 mL (10x), Clear
	Anti-Cyclin D1 (Phospho-Thr286) antibody (Rabbit Polyclonal): 60 μl (100x), Red
	Anti-Cyclin D1 antibody (Rabbit Polyclonal): 60 µl (100x), Purple
	Anti-GAPDH antibody (Mouse Monoclonal): 60 µl (100x), Green
	HRP-Conjugated Anti-Rabbit IgG antibody 12 mL (1x), Glass
	HRP-Conjugated Anti-Mouse IgG antibody 12 mL (1x), Glass

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## Product Details

	Primary antibody Diluent: 12 mL (1x), Clear
	Ready-to-Use Substrate: 12 mL (1x), Brown
	Stop Solution: 12 mL (1x), Clear
	Crystal Violet Solution: 12 mL (1x), Glass
	SDS Solution: 24 mL (1x), Clear
	Adhesive Plate Seals: 4 Seals
Material not included:	The following materials and equipment are NOT provided in this kit but are necessary to successfully conduct the experiment:
Material not included:	The following materials and equipment are NOT provided in this kit but are necessary to successfully conduct the experiment: Microplate reader able to measure absorbance at 450 nm and/or 595 nm for Crystal Violet Cell
Material not included:	successfully conduct the experiment:
Material not included:	successfully conduct the experiment: Microplate reader able to measure absorbance at 450 nm and/or 595 nm for Crystal Violet Cell
Material not included:	successfully conduct the experiment: Microplate reader able to measure absorbance at 450 nm and/or 595 nm for Crystal Violet Cell Staining (Optional)

## Target Details

Target:	Cyclin D1 (CCND1)
Alternative Name:	Cyclin D1 (CCND1 Products)
Molecular Weight:	33729
OMIM:	168461
UniProt:	P24385
Pathways:	PI3K-Akt Signaling, Cell Division Cycle, Mitotic G1-G1/S Phases, ER-Nucleus Signaling

## Application Details

Comment:	Phospho-Cyclin D1 : Human:pThr-286, Mouse:pThr-286, Rat:pThr-286
Plate:	Uncoated
Protocol:	The Colorimetric Cell-Based ELISA Kit allows for the detection of various target proteins and the
	effects that certain stimulation conditions have on target protein expression in different cell
	lines. Qualitative determination of target protein concentration is achieved by an indirect ELISA
	format. In essence, the target protein is captured by target-specific primary (1st) antibodies
	while the HRP-conjugated secondary (2nd) antibodies bind the Fc region of the 1st antibody.
	Through this binding, the HRP enzyme conjugated to the 2nd antibody can catalyze a
	colorimetric reaction upon substrate addition. Due to the qualitative nature of the Cell-Based
	ELISA, multiple normalization methods are described:

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Application Details	
	1) a monoclonal antibody specific for human GAPDH is included to serve as an internal positive
	control in normalizing the target absorbance values.
	2) Following the colorimetric measurement of HRP activity via substrate addition, the Crystal
	Violet whole-cell staining method is used to determine cell density. After staining, the results
	can be analyzed by normalizing the absorbance values to cell amounts, by which the plating
	difference can be adjusted.
	3) If a phosphorylated target is being detected, an antibody against the non- phosphorylated
	counterpart will be provided for normalization purposes. The absorbance values obtained for
	the non-phosphorylated target can be used to normalize the absorbance values for the
	phosphorylated target.
	Cyclin D1 (Phospho-Thr286) Colorimetric Cell-Based ELISA
	The Cyclin D1 (Phospho-Thr286) Cell-Based ELISA Kit is a convenient, lysate-free, high
	throughput and sensitive assay kit that can monitor Cyclin D1 protein phosphorylation and
	expression profile in cells. The kit can be used for measuring the relative amounts of
	phosphorylated Cyclin D1 in cultured cells as well as screening for the effects that various
	treatments, inhibitors (ie. siRNA or chemicals), or activators have on Cyclin D1 phosphorylation.
Reagent Preparation:	Note: Please remember to allow all solutions to warm up to room temperature prior to use.
	1x TBS: 1x TBS is used to wash cells seeded on the plate. 1x TBS can be prepared by adding 1
	volume of 10x TBS provided in the kit to 9 volumes of ddH20.
	Fixing Solution: This solution is NOT provided. Fixing Solution is used to fix cells after cell
	culture. It is prepared by adding formaldehyde to 1x TBS with light mixing. The 4%
	formaldehyde is used for adherent cells and 8% formaldehyde is used for suspension cells and
	loosely attached cells.
	Quenching Buffer: This solution is provided as ready-to-use. Quenching Buffer is used to
	inactivate the endogenous peroxidase activity of the seeded cells.
	Blocking Buffer: This solution is provided as ready-to-use. Blocking Buffer is used to block
	additional binding sites in each well.
	Wash Buffer: This buffer is provided as a 10x solution. 1x Wash Buffer can be prepared by
	adding 1 volume of 10x Wash Buffer provided in the kit to 9 volumes of ddH2O.
	100x Anti-Cyclin D1 (Phospho-Thr286) antibody: This antibody is a rabbit polyclonal antibody.
	This antibody was tested to be specific for the Cyclin D1 protein phosphorylated at Thr286. The
	supplied antibody is a 100x solution. Make 1:100 dilutions in Primary antibody Diluent prior to
	use. The diluted primary antibody can be stored at 4°C for up to two weeks.
	100x Anti-Cyclin D1 antibody: This antibody is a rabbit polyclonal antibody. This antibody was
	roox/ interesting of a new and body in a rabbit polycional anabody. This anabody was

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	1:100 dilutions in Primary antibody Diluent prior to use. The diluted primary antibody can be
	stored at 4°C for up to two weeks.
	100x Anti-GAPDH antibody: This antibody is a mouse monoclonal antibody. This antibody was
	tested to be specific for GAPDH. The supplied antibody is a 100x solution. Make 1:100 dilutions
	in Primary antibody Diluent prior to use. The diluted primary antibody can be stored at 4°C for
	up to two weeks.
	HRP-Conjugated Anti-Rabbit IgG antibody: This solution is provided as ready-to-use. HRP-
	Conjugated Anti-Rabbit IgG antibody is used as the secondary antibody to detect the target-
	bound, primary rabbit antibodies.
	HRP-Conjugated Anti-Mouse IgG antibody: This solution is provided as ready-to-use. HRP-
	Conjugated Anti-Mouse IgG antibody is used as the secondary antibody to detect the target-
	bound, primary mouse antibodies.
	Primary antibody Diluent: This solution is provided as ready-to-use. Use this solution to dilute
	the provided antibodies.
	Ready-to-Use Substrate: This solution is provided as ready-to-use. Ready-to-Use Substrate
	must be warmed to room temperature before use. Keep away from light as this solution is light-
	sensitive.
	Stop Solution: This solution is provided as ready-to-use. Stop Solution must be handled with
	caution as it contains 2 N Sulfuric Acid (H2SO4) and is corrosive. Wear eye protection and
	gloves when handling.
	Crystal Violet Solution: This solution is provided as ready-to-use. Crystal Violet is an intense
	stain used to stain cell nuclei. Avoid contact with skin and clothing.
	SDS Solution: This solution is provided as ready-to-use. SDS is used to solubilize the Crystal
	Violet in preparation for cell staining. Store this solution at room temperature or warm up to
	room temperature if stored at 4°C.
Assay Procedure:	Note: Please read the whole manual before performing the experiment.
	1) Seed 200 $\mu$ L of 20,000 adherent cells in culture medium in each well of a 96-well plate. The
	plates included in the kit are sterile and treated for cell culture. For suspension cells and loosely
	attached cells, coat the plates with 100 $\mu L$ of 10 $\mu g/mL$ Poly-L-Lysine (not included) to each
	well of a 96-well plate for 30 minutes at 37°C prior to adding cells.
	2) Incubate the cells for overnight at 37°C, 5% CO2.
	3) Treat the cells as desired.
	4) Remove the cell culture medium and rinse with 200 $\mu L$ of 1x TBS, twice.
	5) Fix the cells by incubating with 100 $\mu L$ of Fixing Solution for 20 minutes at room
	temperature. The 4% formaldehyde is used for adherent cells and 8% formaldehyde is used for

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6) Remove the Fixing Solution and wash the plate 3 times with 200 µL 1x Wash Buffer for five minutes each time with gentle shaking on the orbital shaker. The plate can be stored at 4°C for a week. Note: For all wash steps, tap the plate gently on absorbent papers to remove the solution completely.

7) Add 100 µL Quenching Buffer and incubate for 20 minutes at room temperature.
8) Wash the plate 3 times with 1x Wash Buffer for 5 minutes at a time, with gentle shaking on the shaker.

9) Add 200 µL of Blocking Buffer and incubate for 1 hour at room temperature. 10) Wash 3 times with 200 µL of 1x Wash Buffer for 5 minutes at a time, with gentle shaking on the shaker. 11) Add 50 µL of 1x primary antibodies (Anti-Cyclin D1 (Phospho- Thr286) antibody, Anti-Cyclin D1 antibody and/or Anti-GAPDH antibody) to the corresponding wells, cover with Parafilm and incubate for 16 hours (overnight) at 4°C. If the target expression is known to be high, incubate for 2 hours at room temperature with gentle shaking on the shaker.

12) Wash 3 times with 200  $\mu$ L of 1x Wash Buffer for 5 minutes at a time, with gentle shaking on the shaker.

13) Add 50 µL of 1x secondary antibodies (HRP-Conjugated Anti- Rabbit IgG antibody and/or HRP-Conjugated Anti-Mouse IgG antibody) to corresponding wells and incubate for 1.5 hours at room temperature with gentle shaking on the shaker. Note: Add HRP-Conjugated Anti-Rabbit IgG antibody to the wells incubated with Anti-Cyclin D1 (Phospho-Thr286) antibody (rabbit, polyclonal) and/or Anti-Cyclin D1 (rabbit, polyclonal) and add HRP- Conjugated Anti-Mouse IgG antibody to the wells incubated with Anti-GAPDH antibody (mouse, monoclonal).

14) Wash 3 times with 200  $\mu$ L of 1x Wash Buffer for 5 minutes at a time, with gentle shaking on the shaker.

15) Add 50 µL of Ready-to-Use Substrate to each well and incubate for 30 minutes at room temperature in the dark with gentle shaking on the shaker. Note: Ready-to-Use Substrate is a light-sensitive reagent. Keep away from light.

16) Add 50 µL of Stop Solution to each well and read OD at 450 nm immediately using the microplate reader. Optional: Crystal Violet Cell Staining Crystal Violet binds to cell nuclei and gives absorbance readings proportional to cell counts at 595 nm.

17) After finishing reading the absorbance at 450 nm, wash the plate twice with 200  $\mu$ L of Wash Buffer and twice with 200  $\mu$ L of 1x TBS for 5 minutes each. Tap the plates on paper towel to remove the excess liquid. Let plate air dry for 5 minutes at room temperature.

18) Add 50 µL of Crystal Violet Solution to each well, incubate for 30 minutes at room

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	temperature on the shaker. Note: Crystal Violet is an intense stain. Avoid contact with skin and
	clothing.
	19) Flick the plate to remove Crystal Violet Solution, rinse the plate by filling the wells with
	running tap water, and wash the plate with 200 $\mu$ L of 1x TBS 3 times, 5 minutes each with
	gently shaking on the shaker.
	20) Add 100 $\mu L$ of SDS Solution into each well and incubate on the shaker at room temperature for 1 hour.
	21) Read absorbance at 595 nm with microplate reader. If absorbance is too high, the
	solubilized Crystal Violet Solution can be diluted 10 times with H2O on a separate 96-well plate.
Calculation of Results:	Anti-Cyclin D1 antibody normalization: The OD values obtained for the phosphorylated target
	protein can be normalized using the OD values obtained for the non-phosphorylated target
	protein via the proportion, OD450 (Anti-Cyclin D1 P-Thr286 antibody)/OD450 (Anti-Cyclin D1
	antibody).
	GAPDH normalization: The OD450 values obtained for the target protein (phosphorylated and
	non- phosphorylated) can be normalized using the OD450 values obtained for GAPDH.
	Crystal Violet Staining normalization: The measured OD450 readings can be normalized using
	the OD595 values via the proportion, OD450/OD595.
Assay Precision:	Cell-Based
	This ELISA kit is intended for research purposes only, NOT for diagnostic or clinical procedures
	of any kind.
	Materials included in this kit should NOT be used past the expiration date on the kit label.
	Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or
	substrates from any other kits.
	Variations in pipetting technique, washing technique, operator laboratory technique, kit age,
	incubation time or temperature may cause differences in binding affinity of the materials
	provided.
	The assay is designed to eliminate interference and background by other cellular
	macromolecules or factors present within any biological samples. However, the possibility of
	background noise cannot be fully excluded until all factors have been tested using the assay kit
Restrictions:	For Research Use only
Handling	
Precaution of Use:	Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.

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	Fixing Solution contains formaldehyde. Formaldehyde is known to be a highly toxic reagent.
	Personal protection is strongly recommended while working with this chemical.
	Stop Solution contains 2 N Sulfuric Acid (H2SO4) and is an extremely corrosive agent. Please
	wear proper eye, hand and face protection when handling this material. When the experiment is
	finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop
	Solution prior to disposing the plate or strips.
	Crystal Violet is an intense stain reagent. Avoid contact with clothing.
Storage:	4 °C
5	
Storage Comment:	Upon receipt, the kit should be stored at 4°C. The un-opened kit will be stable for up to 6
	Upon receipt, the kit should be stored at 4°C. The un-opened kit will be stable for up to 6 months from the date of shipment if stored at 4°C. Diluted Anti-Cyclin D1 (Phospho-Thr286)
	months from the date of shipment if stored at 4°C. Diluted Anti-Cyclin D1 (Phospho-Thr286)
	months from the date of shipment if stored at 4°C. Diluted Anti-Cyclin D1 (Phospho-Thr286) antibody, Anti-Cyclin D1 antibody and diluted Anti-GAPDH antibody can each be stored at 4°C
	months from the date of shipment if stored at 4°C. Diluted Anti-Cyclin D1 (Phospho-Thr286) antibody, Anti-Cyclin D1 antibody and diluted Anti-GAPDH antibody can each be stored at 4°C for up to two weeks. HRP-Conjugated Anti-Rabbit IgG antibody and HRP-Conjugated Anti-