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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The CDK12/ Cyclin K Kinase Assay Kit is designed to measure the activity of complex CDK12/ Cyclin K for screening and profiling applications using luminescent Kinase-Glo® MAX as a detection reagent. The CDK12/ Cyclin K Kinase Assay Kit comes in a convenient 96-well format, with enough purified CDK12/ Cyclin K, CDK12/ Cyclin K Substrate, ATP, and kinase assay buffer for 96 enzyme reactions.

Background

The CDK12/Cyclin K complex comprises human CDK12 (Cyclin Dependent Kinase 12) and human Cyclin K. Cyclin-dependent kinase (CDK) complexes have been implicated in the regulation of transcription. The CDK12/ Cyclin K complex regulates phosphorylation of Serine 2 in the C-terminal domain of RNA polymerase II, which is responsible for productive transcriptional elongation and synthesis of full-length mature mRNAs. This complex contributes to the maintenance of genome stability and plays a role in embryonic development as well as cancer.

Applications

Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

Catalog #	Name	Amount	Storage
100998	CDK12/ Cyclin K*	50 µg	-80°C
79334	5x Kinase assay buffer	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
78299	CDK12/Cyclin K Substrate (10 mg/ml)	200 µl	-20°C
79696	96-well plate, white	1	Room Temp.

**The initial concentration of CDK12 is lot-specific and will be indicated on the tube containing the enzyme.*

Materials Required but Not Supplied

Name	Catalog #
Kinase-Glo® MAX	Promega #V6073
Dithiothreitol (DTT 0.5 M)	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw 5x Kinase assay buffer, ATP (500 µM), and CDK12/ Cyclin K Substrate (10 mg/ml). Add DTT (0.5 M) to 5x Kinase assay buffer to make a 10 mM concentration; e.g., add 20 µl of 0.5 M DTT to 1 ml 5x Kinase assay buffer).
2. Dilute 5x Kinase assay buffer to 1x Kinase assay buffer with distilled water.
3. Prepare the master mixture (25 µl per well): N wells x (6 µl 5x Kinase assay buffer + 1 µl ATP (500 µM) + 2 µl CDK12/ Cyclin K Substrate (10 mg/ml) + 16 µl distilled water). Add 25 µl to every well.
4. Prepare the Test Inhibitor by diluting test compound (in 100% DMSO) 1:10 with distilled water. If the compound is soluble in water, dissolve in water to make a solution that is 10x the desired final concentration.
   Add 5 µl of Test Inhibitor to each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of 10% DMSO aqueous (or 5 µl of water if compound was dissolved in water). Note: Keep DMSO concentration of the Test Inhibitor at ≤10%, as the final DMSO concentration in the reaction should be ≤1%.
5. Prepare 3 ml of 1x Kinase assay buffer by mixing 600 µl of 5x Kinase assay buffer with 2400 µl of water. Three ml of 1x Kinase assay buffer is sufficient for 100 reactions.
6. To the wells designated as "Blank," add 20 µl of 1x Kinase assay buffer.
7. Thaw CDK12/ Cyclin K complex on ice. Upon first thaw, briefly spin the tube to recover the full content. Calculate the amount of CDK12/ Cyclin K required for the assay and dilute the enzyme to 25 ng/µl with 1x Kinase assay buffer. Store the remaining undiluted enzyme in aliquots at -80°C. *Note:* CDK12/ Cyclin K *enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
8. Initiate the reaction by adding 20 µl of diluted CDK12/ Cyclin K complex to the wells designated “Positive Control” and "Test Inhibitor." Incubate at 30°C for 90 minutes.

Component	Blank	Positive Control	Test Inhibitor
Master mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
10 % DMSO (aqueous)	5 µl	5 µl	-
1x Kinase Buffer 1	20 µl	-	-
CDK12/ Cyclin K (25 ng/µl)	-	20 µl	20 µl
Total	50 µl	50 µl	50 µl

1. Thaw Kinase-Glo® MAX reagent.
2. After the 90 minutes reaction, add 50 µl of Kinase-Glo® MAX reagent to each well. Cover the plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
3. Read using a luminometer or microtiter-plate capable of reading chemiluminescence. The “Blank” value is subtracted from all readings.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry. To properly read chemiluminescence, make sure that the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

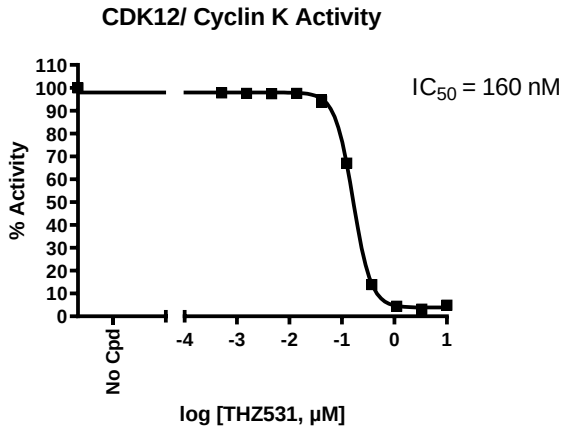

Figure 1: Inhibition of CDK12/ Cyclin K *by increasing concentrations of THZ 531 (Selleck Chemicals, #S6595), measured using the CDK12/Cyclin K Kinase Assay Kit (BPS Bioscience #78298). Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

General considerations

"Blank" Control: The "Blank" control is important to determine the background absorbance in the assay.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

1. Zhang, T., Kwiatkowski, N., Olson, C. *et al.* Covalent targeting of remote cysteine residues to develop CDK12 and CDK13 inhibitors. *Nat Chem Biol* **12,** 876–884 (2016). https://doi.org/10.1038/nchembio.2166
2. Bösken CA, Farnung L, Hintermair C, Merzel Schachter M, Vogel-Bachmayr K, Blazek D, Anand K, Fisher RP, Eick D, Geyer M. The structure and substrate specificity of human Cdk12/Cyclin K. Nat Commun. 2014 Mar 24;5:3505. doi: 10.1038/ncomms4505. PMID: 24662513; PMCID: PMC3973122.

Related Products

Products	*Catalog #*	*Size*
CDK12/Cyclin K, GST-tags	101235	20 µg
CDK13/Cyclin K, GST-Tags	101128	5 µg / 10 µg
CDK11A/CyclinD3, GST-tag	100595	20 µg
CDK1 Assay Kit	79597	96 reactions
CDK2 Assay Kit	79599	96 reactions
CDK4 Assay Kit	79674	96 reactions
CDK5 Assay Kit	79600	96 reactions
CDK7 Assay Kit	79603	96 reactions
CDK9 Assay Kit	79628	96 reactions