Tribioscience

CDK4/Cyclin D Activity Fluorometric Assay

(Catalog: TBS2127, 100 Assays, Store at -20ºC)

DESCRIPTION

Cyclin-Dependent Kinase 4 (CDK4) is a critical serine/threonine kinase that regulates the G1/S cell cycle checkpoint. The active CDK4/Cyclin D complex mono-phosphorylates the retinoblastoma-associated protein (Rb), disrupting its interaction with the E2F transcription factor. The subsequent release of E2F activates the transcription of genes essential for DNA replication and S-phase transition. Aberrant CDK4 expression or mutations bypass the normal cell cycle checkpoints, driving uncontrolled cell proliferation and oncogenesis.

Tribioscience's CDK4/Cyclin D Activity Assay is designed to be a flexible, accurate, simple, and time-saving method in which CDK4/Cyclin D catalyzes Rb into P-Rb and ADP, resulting in an intermediate formation through a coupled enzyme reaction. It reacts with a probe to generate a fluorometric shift measured at Ex/Em = 530/590 nm, where fluorescence intensity directly correlates with CDK4/Cyclin D enzymatic activity.

SYNONYMS

CMM3, Cyclin D-CDK4, PSK-J3, cyclin dependent kinase 4.

APPLICATIONS

CDK4/Cyclin D activity in cells and various biological samples.

KIT CONTENTS

Name	Size (100 tests)
Assay Buffer	20 mL
CDK4 Enzyme Mix	14 µL
CDK4 Substrate	50 µL
Co-substrate	14 µL
Assay Cofactor	40 µL
Assay Developer	35 µL
CDK4 Probe	240 µL
CDK4/Cyclin D Positive Control	17 µL (10x)
ADP standard (5 mM)	50 µL

STORAGE AND HANDLING

Store the kit at -20°C. The shelf life is 12 months.

ASSAY PROTOCOL

Except Enzyme, warm all the components to room temperature before use. Briefly centrifuge all small vials prior to opening.

1. Sample Preparation:

Tissue (1-10 mg) or cells (1 x 10^6) can be lysed in 100 µL of Assay Buffer. Centrifuge ice cold at 15,000 x g for 2 minutes to pellet insoluble materials and collect supernatant. For more accurate assay, the sample should be quickly frozen using liquid N_2 or dry ice if it is to be assayed later.

2. Standard Curve Preparations:

2.1 Label 1.5mL tubes 1-8 for a standard curve preparation displayed as Table 1.

Add 288µL of 1x Assay Buffer to Std 1 and 150µL to Std 2-8.

2.2 Add 12 µL of 5 mM ADP Stock solution to Std1 and fully mix,

2.3 Carry out a 2x serial dilution sequentially for Std 2-7 by transferring 200µL of Std1 to Std2 and afterwards. Leave Std 8 as the 0 standard (the assay buffer alone as blank). The standards concentrations are 200, 100, 50, 25, 12.5, 6.25, 3.13, and 0 µM for Std 1-8.

Table1: Assay Standard Preparation

Tubes	ADP (µL)	Buffer	ADP (µM)
1	12 µL 5 mM stock	288 µL	200
2	150 µL of Tube#1	150 µL	100
3	150 µL of Tube#2	150 µL	50
4	150 µL of Tube#3	150 µL	25
5	150 µL of Tube#4	150 µL	12.5
6	150 µL of Tube#5	150 µL	6.25
7	150 µL of Tube#6	150 µL	3.13
8	0	150 µL	0

3. Positive Control Preparation: Prepare CDK4/Cyclin D positive control (1x) by adding15 µL of CDK4/Cyclin D positive control (10x) + 135 µL assay buffer;

4. Load Sample: Add 50 µL/well standards or samples or CDK4/Cyclin D positive control (1x) to 96-well plate (Note: use black plate for fluorometric assay).

5. CDK4/Cyclin D Reaction Mix1:

Prepare mix1 for 100 wells test by adding 35 µL of CDK4 substrate to 1965 µL of assay buffer. Transfer 20 µL Mix1 to each well except standards. Add 20 µL assay buffer to each standard instead of Mix1.

6. Incubate the plate at 37℃ for 1 hour as T1.

7. CDK4/Cyclin D Reaction Mix2:

Prepare 3000 µL of reaction mix2 for 100 wells test as Table 2:

Table 2: Reaction Mix Preparation

Reaction Components	Volume
Assay Buffer	2722 µL
Co-substrate	10 µL
Assay Cofactor	29.5 µL
CDK4 Enzyme mix	11.5 µL
Assay developer	27 µL
CDK4 probe	200 µL
Final Volume (µL)	**3000 µL**

Add 30 µL of the CDK4/Cyclin D Reaction Mix2 to each well containing the ADP Standards, CDK4/Cyclin D Positive Control, and test samples. Tap the plate lightly to mix. Protect the plate from light during incubation.

8. Measurement:

Measure the absorbance value at fluorescence at Ex/Em = 530/590 nm in kinetic model for 15-30 min after adding mix2 as T2 at 37°C (Note: the incubation time is dependent on the CDK4/Cyclin D activity in the samples).

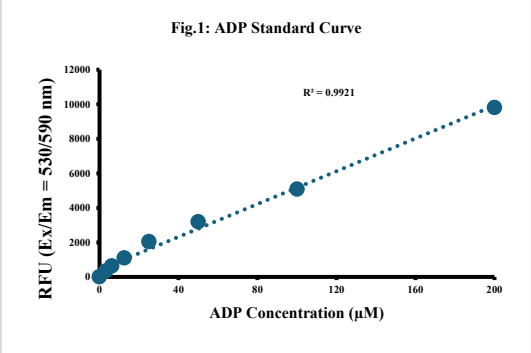
9. Calculation:
Correct background by subtracting the value of the 0 standard (blank) from all readings to get ΔRFU. Plot the standard curve. Apply the corrected sample value to the standard curve to get B µM of ADP generated by CDK4/Cyclin D during the reaction time. (e.g. The formular on standard curve is Y=mB+c, Y as sample ΔRFU, then **B**=(Y-c)/m, c: the Y-intercept, a: the slope of the line).

Sample CDK4/Cyclin D Activity = N* B/(T*V) = µM/µl or = U/µl.

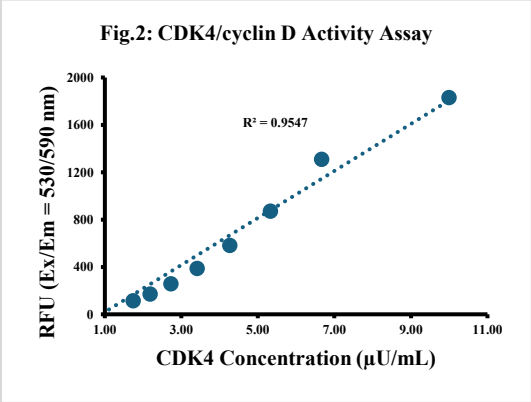
Where: B=ADP amount from Standard Curve (µM)
V= Sample volume in the reaction well (µL)
N= Dilution Factor
T= incubation time (T1+T2 total minutes)

Unit Definition: One unit (U) is the amount of enzyme that will generate 1.0 µmol of ADP per minute at pH7.5 at 37 ℃.

8. Typical Standard Curve for Fluorometric Assays:
The typical standard curves are shown in Fig. 1. The Typical standard curves are only used as a reference for assay. It cannot be used for real standard curve for assay.



The enzyme activity assay curves are shown in Fig. 2



RELATED PRODUCTS
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ETNK Fluorometric Assay (TBS2209))
EGFR Activity Assay (TBS2048)
LDH Cytotoxicity Assay (TBS2002)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay (TBS2020)
XTT Cell Viability Assay (TBS2021)
Cell Count Kit -8 (TBS2022)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
NAD/NADH Colorimetric Assay (TBS2029)
Caspase-3 Colorimetric Assay (TBS2030)
Pyruvate Colorimetric Assay (TBS2023C)
Glycerol Colorimetric Assay (TBS2204C)
Triglyceride Colorimetric Assay (TBS2205C)
NNMT Inhibitor Screening Assay (TBS2097)
NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102
Cytochrome c Reductase Activity Assay (TBS2116)
Mitochondria Complex 1 Activity Assay (TBS2017)
Mitochondria Oxidase Activity (TBS2105)
Mitochondrial Membrane Potential Assay (TBS2049)
Cytochrome C Oxidase (Complex IV) Activity Assay (TBS2115)

This product is for research only.