Store at –20°C

#1098

cdc2 (Tyr15) Biotinylated Peptide

☑ 1.25 ml at 12 µM

Cell Signaling TECHNOLOGY®

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New 03/07

Description: This biotinylated peptide contains the residues surrounding Tyr15 of cdc2. It was generated for the use in CST's HTScan® kinase assay kits, but may also serve as a substrate in other heterogeneous or homogeneous kinase assays.

Peptide Core Sequence: EGTY*GVV

Molecular Weight: 1667 daltons

Quality Control: The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

Directions for Use: The phosphorylated form of the peptide can be detected with the Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411. Sample kinase assay protocol is attached.

Storage: Supplied in 0.0001% DMSO. Store at -20°C.

Companion Products:
Tyrosine Kinase Substrate Screening Kit #7450

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

Lyn Kinase #7610

Protocol for Tyrosine Kinase Assay

IMPORTANT: Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-Tyrosine mAb (P-Tyr-100) #9411
5. Kinase Buffer (4X) #9805
6. ATP (10 mM) #9804
7. DTT (1.25M)
8. Kinase (See companion products)
9. DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
10. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
11. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

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B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6-12 µM substrate peptide. Adjust the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3-6 µM).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 10 ml of DTT (1.25M) to 2.5 ml of 4X HTScan® tyrosine kinase buffer (240 mM HEPES pH 7.5, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 mM Na_3VO_4) to make 4xDTT/Kinase buffer.
5. Dilute enzyme in 1.25 ml of 4X DTT/Kinase buffer to make 4X reaction cocktail ([enzyme]=0.8-8.0 ng/µl in 4X DTT/Kinase buffer).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

60 mM HEPES (pH7.5)
5 mM $MgCl_2$
5 mM $MnCl_2$
3 mM Na_3VO_4
1.25 mM DTT
200 µM ATP
1.5-3 µM peptide
10-100 ng kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody (Phospho-Tyrosine mAb (P-Tyr-100) #9411) in PBS/T with 1% BSA. *Add 100 µl/well primary antibody.
13. Incubate at 37°C for 120 minutes.
14. Wash three times with 200 µl/well PBS/T.
15. Dilute Europium-labeled secondary antibody in PBS/T with 1% BSA. **Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

***Recommended antibody dilution factor:**
Primary antibody:
Mouse mAb: 1:500
Rabbit mAb or polyclonal antibody: 1:1000

****Secondary antibody:**
DELFIA® Europium-labeled Anti-mouse IgG: 1:500
DELFIA® Europium-labeled Anti-rabbit Antibody: 1:1000