

Phospho-KDR (Tyr996) Biotinylated Peptide

1.25 ml at 6 µM

new 08/07



Cell Signaling
TECHNOLOGY®

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Description: This biotinylated peptide contains the residues surrounding Tyr996 of KDR. The tyrosine residue in the peptide corresponding to Tyr996 has been chemically phosphorylated in the course of peptide synthesis. This phosphopeptide was generated for use as a positive control in CST's HTScan® c-Kit Kinase Assay Kit #7755, but it may also serve as a positive control in other heterogeneous or homogeneous kinase assays.

Peptide Core Sequence: EDLY*KDF

Molecular Weight: 2244 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 mAb (P-Tyr-100) #9411. Sample kinase assay protocol is attached.

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

Companion Products:

HTScan® c-Kit Kinase Assay Kit #7755

c-Kit Kinase #7754

c-Kit D816V Kinase #7924

c-Kit T670I Kinase #7922

KDR (Tyr996) Biotinylated Peptide #1364

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

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)

B Suggested Protocol for 100 Assays

1. Add 100 μ l 10 mM ATP to 1.25 ml 6-12 μ M substrate peptide. Adjust the mixture with dH₂O to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 μ M, [substrate] = 3-6 μ M).
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 μ l of DTT (1.25M) to 2.5 ml of 4X HTScan® tyrosine kinase buffer (240 mM HEPES pH 7.5, 20 mM MgCl₂, 20 mM MnCl₂, 12 μ M Na₃VO₄) 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/ μ l in 4X DTT/Kinase buffer).
6. Add 12.5 μ l of the 4X reaction cocktail to 12.5 μ l/well of prediluted compound of interest (usually around 10 μ M) and incubate for 5 minutes at room temperature.
7. Add 25 μ l of 2X ATP/substrate cocktail to 25 μ l/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 μ l Reaction

60 mM HEPES (pH7.5)
5 mM MgCl₂
5 mM MnCl₂
3 μ M Na₃VO₄
1.25 mM DTT
200 μ M ATP
1.5-3 μ M peptide
10-100 ng kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 μ l/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 μ l of each reaction to a 96-well streptavidin-coated plate containing 75 μ l dH₂O/well and incubate at room temperature for 60 minutes.
11. Wash three times with 200 μ l/well PBS/T.
12. Dilute primary antibody (Phospho-Tyrosine mAb (P-Tyr-100) #9411) in PBS/T with 1% BSA. *Add 100 μ l/well primary antibody.
13. Incubate at 37°C for 120 minutes.
14. Wash three times with 200 μ l/well PBS/T.
15. For DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

DELFIA® is a registered trademark of PerkinElmer Life Sciences

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 μ l/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 μ l/well PBS/T.
5. Add 100 μ l/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a Time Resolved Fluorescent plate reader using the following settings;
 - a. Excitation Filter: 340 nm
 - b. Emission Filter: 615 nm
 - c. Delay**: 400 μ s
 ** Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 μ l/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 μ l/well PBS/T.
5. Add 100 μ l/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 μ l/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

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

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com