

eNOS (Ser1177) Biotinylated Peptide

1.25 ml at 6 µM



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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 serine 1177 of eNOS. It was generated for 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: RTQS*FSL

Molecular Weight: 2431 daltons

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

Notes on Use: The phosphorylated form of the peptide can be detected with the Phospho-eNOS (Ser1177) (C9C3) Rabbit mAb #9571. Sample kinase assay protocols can be found on corresponding kinase assay kit data sheets (see Companion Products).

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

Companion Products:

HTScan® Akt1 Kinase Assay Kit #7501

HTScan® Akt2 Kinase Assay Kit #7504

HTScan® Akt3 Kinase Assay Kit #7507

Serine/Threonine Kinase Substrate Screening Kit #7400

Phospho-eNOS (Ser1177) (C9C3) Rabbit mAb #9570

Protocol for Serine/Threonine 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. Kinase Buffer (10X) #9802
5. ATP (10 mM) #9804
6. DELFIA® Europium-labeled Anti-rabbit Antibody (PerkinElmer Life Sciences #AD0105) or DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
7. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
8. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)
9. Active kinase (See companion products)
10. Primary antibody (See companion products)

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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 dH₂O 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 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM MgCl₂, 1 mM Na₃VO₄, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH₂O to make 2.5 ml 4X reaction buffer.
5. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail (enzyme)=0.8-8.0 ng/µl in 4X reaction cocktail).
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

- 25 mM Tris-HCl (pH7.5)
 - 10 mM MgCl₂
 - 5 mM β-glycerophosphate
 - 0.1 mM Na₃VO₄
 - 2 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 dH₂O/well and incubate at room temperature for 60 minutes.
 11. Wash three times with 200 µl/well PBS/T.
 12. Dilute primary antibody 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