

#1416

Store at -20°C

## EGFR (Thr693) Biotinylated Peptide

1.25 ml at 12 µM



Cell Signaling  
TECHNOLOGY®

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New 02/07

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 Thr693 of EGF receptor. 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:** EPLT\*PSG

**Molecular Weight:** 2798 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-MAPK Substrates (PXT) (46G11) Rabbit mAb #4391. Sample kinase assay protocol is attached.

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

**Companion Products:**

Serine/Threonine Kinase Substrate Screening Kit #7400

Phospho-MAPK Substrates (PXT) (46G11) Rabbit mAb #4391

Erk1 Kinase #7416

## 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 dH<sub>2</sub>O 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 MgCl<sub>2</sub>, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH<sub>2</sub>O 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 MgCl<sub>2</sub>
  - 5 mM β-glycerophosphate
  - 0.1 mM Na<sub>3</sub>VO<sub>4</sub>
  - 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 dH<sub>2</sub>O/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