Store at -20°C

#1512

Phospho-EGFR (Thr693) Biotinylated Peptide

☑ 1.25 ml at 6 µM

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

New 02/08

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 EGFR. The threonine residue in the peptide has been chemically phosphorylated in the course of peptide synthesis. This phosphopeptide was generated for use as a positive control in kinase assays (Erk1 kinase #7416, Erk2 kinase #7591), but it may also serve as a positive control in other heterogeneous or homogeneous kinase assays.

Peptide Core Sequence: EPLT*PSG

Molecular Weight: 2878 daltons

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

Directions for Use: This phosphorylated peptide can be detected with the Phospho-MAPK Substrates (PXTP) (46G11) Rabbit mAb #4391. A sample kinase assay protocol is attached.

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

Companion Products:

Erk1 Kinase #7416

Erk2 Kinase #7591

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

EGFR (Thr693) Biotinylated Peptide #1416

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. Active kinase (See companion products)
7. Primary antibody (See companion products)

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 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 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 $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
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_2O/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.(1:500 dilution for mouse mAb or 1:1000 dilution for rabbit mAb or polyclonal antibody)
13. Incubate at 37°C for 120 minutes.
14. Wash three times with 200 µl/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 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/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 µs

++ 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 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/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