Store at –20°C

#1046

IRS-1 (Ser1101) Biotinylated Peptide

☑1.25 ml at 6 µM

Cell Signaling TECHNOLOGY®

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New 12/06

Description: This biotinylated peptide 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: RHSS*ETF

Molecular Weight: 1932 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-IRS-1 (Ser1101) Antibody #2385. 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-IRS-1 (Ser1101) Antibody #2385

RSK1 Kinase #7398

RSK2 Kinase #7404

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_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.
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