Store at –20°C

#1585

Poly (Glu-Tyr) Biotinylated Peptide

☑1.25 ml at 6 µM

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Description: This biotinylated peptide contains glutamic acid (Glu) and tyrosine (Tyr) at a ratio of 4:1 (Glu:Tyr). This biotinylated peptide was generated for use as substrate in a tyrosine kinase assays, and also can serve as a positive control in tyrosine phosphatase assays.

Peptide Core Sequence: Glu:Tyr 4:1

Molecular Weight: 3250 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 Mouse mAb (P-Tyr-100) #9411. Sample kinase and phosphatase assay protocols are attached.

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

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

Phospho-Poly Glu-Tyr Biotinylated Peptide #1586

#1585

Protocol for Tyrosine Phosphatase/Kinase

Phosphatase Assay:

A Additional Solutions and Reagents (Not included)

- **Phosphatase Buffer (5X)**
  125 mM HEPES, pH 7.2
  250 mM NaCl
  12.5 mM EDTA
- Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586
- DTT (1.25 M)
- BSA (65 ng/µl)
- Stop solution (2N NaOH)
- Wash Buffer: 1X, PBS 0.05% Tween-20 (PBS/T)
- Phospho-Tyrosine mAb (P-Tyr-100) #9411

B Suggested Protocol for 100 Assays

1. Prepare fresh batches of 1X Phosphatase Assay Buffer by diluting the Phosphatase Buffer (5X) at a 1:4 ratio with a solution containing 5 mM DTT and 65 ng/µl BSA.
2. Dilute 1 mM Phospho-Poly EY (20) Biotinylated Peptide substrate solution to 3 µM with 1X Phosphatase Assay Buffer.
3. Thaw enzyme on ice.
4. Dilute phosphatase protein to 0.2 to 2.0 ng/µl with 1X Phosphatase Assay Buffer.
5. To start the reaction combine 25 µl diluted phosphatase solution and 25 µl substrate (3 µM). Incubate at 37°C for 5 to 60 minutes.

Final Assay Conditions for a 50 µl Reaction

25 mM HEPES, pH 7.2
50 mM NaCl
2.5 mM EDTA
5 mM DTT
65 ng/µl BSA
1.5 µM Phospho-Poly (Glu-Tyr) Biotinylated Peptide
0.1 to 1.0 ng/µl phosphatase

6. Terminate reaction by adding 50 µl of 2N NaOH Stop Solution to each reaction well.
7. For DELFIA® or colorimetric ELISA detection methods please use the protocols described to the right.

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 8.0
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 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 10 µl of DTT (1.25M) to 2.5 ml of 4X HTScan® tyrosine kinase buffer (240 mM HEPES pH 7.5, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 µM Na_3VO_4) 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/µl in 4X DTT/Kinase buffer).
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

60 mM HEPES pH 7.5
5 mM $MgCl_2$
5 mM $MnCl_2$
3 µM Na_3VO_4
1.25 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 8.0) 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 (Phospho-Tyrosine mAb (P-Tyr-100) #9411) 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. 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.

For any questions please contact:
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