

# FHIT (Tyr114) Biotinylated Peptide

1.25 ml at 6µM



Cell Signaling  
TECHNOLOGY®

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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 Tyr114 of FHIT. 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:** DSIY\*EEL

**Molecular Weight:** 2150 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 assay protocols can be found on corresponding Kinase Assay Kit data sheets (see Companion Products).

**Entrez-Gene ID** #2272

**Swiss-Prot Acc.** #P49789

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

**Companion Products:**

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

HTScan® Lck Kinase Assay Kit #7758

Tyrosine Kinase Substrate Screening Kit #7450

## Protocol for Tyrosine 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. 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  $\mu$ l 10 mM ATP to 1.25 ml 6–12  $\mu$ M substrate peptide. Adjust the mixture with dH<sub>2</sub>O to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400  $\mu$ M, [substrate] = 3–6  $\mu$ M).
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  $\mu$ l of DTT (1.25M) to 2.5 ml of 4X HTScan® tyrosine kinase buffer (240 mM HEPES pH 7.5, 20 mM MgCl<sub>2</sub>, 20 mM MnCl<sub>2</sub>, 12  $\mu$ M Na<sub>3</sub>VO<sub>4</sub>) 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/ $\mu$ l in 4X DTT/Kinase buffer).
6. Add 12.5  $\mu$ l of the 4X reaction cocktail to 12.5  $\mu$ l/well of prediluted compound of interest (usually around 10  $\mu$ M) and incubate for 5 minutes at room temperature.
7. Add 25  $\mu$ l of 2X ATP/substrate cocktail to 25  $\mu$ l/well preincubated reaction cocktail/compound.

#### Final Assay Conditions for a 50 $\mu$ l Reaction

60 mM HEPES (pH7.5)  
 5 mM MgCl<sub>2</sub>  
 5 mM MnCl<sub>2</sub>  
 3  $\mu$ M Na<sub>3</sub>VO<sub>4</sub>  
 1.25 mM DTT  
 200  $\mu$ M ATP  
 1.5–3  $\mu$ M peptide  
 10–100 ng kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50  $\mu$ l/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25  $\mu$ l of each reaction to a 96-well streptavidin-coated plate containing 75  $\mu$ l dH<sub>2</sub>O/well and incubate at room temperature for 60 minutes.
11. Wash three times with 200  $\mu$ l/well PBS/T.
12. Dilute primary antibody (Phospho-Tyrosine mAb (P-Tyr-100) #9411) in PBS/T with 1% BSA. \*Add 100  $\mu$ l/well primary antibody.
13. Incubate at 37°C for 120 minutes.
14. Wash three times with 200  $\mu$ l/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  $\mu$ l/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. \*Wash five times with 200  $\mu$ l/well PBS/T.
5. Add 100  $\mu$ l/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  $\mu$ s
 \*\* 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  $\mu$ l/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. \*Wash five times with 200  $\mu$ l/well PBS/T.
5. Add 100  $\mu$ l/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100  $\mu$ l/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