

HTScan® HER4/ErbB4 Kinase Assay Kit

100 assays
 (96 Well Format)



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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Products Included	Products #	Kit Quantity
Phospho-Tyrosine Mouse mAb (P-Tyr-100)	9411	30 µl
HTScan® Tyrosine Kinase Buffer (4X)	9805	15 ml
DTT (1000X, 1.25 M)		80 µl
ATP (10 mM)	9804	1 ml
PYK2 (Tyr402) Biotinylated Peptide	1315	1.25 ml
HER4/ErbB4 Kinase (recombinant, human)	7724	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human HER4/ErbB4 kinase. It includes active HER4/ErbB4 kinase (supplied as a GST fusion protein), a biotinylated peptide substrate and a phospho-tyrosine antibody for detection of the phosphorylated form of the substrate peptide.

Peptide Core Sequence: DIY*AE

Molecular Weights: Peptide substrate, Biotin-peptide: 2,166 Daltons. GST-HER4/ErbB4 Kinase: 102 kDa.

Background: The HER/ErbB receptor tyrosine kinase family has been implicated in normal development, cardiac function and cancer (1,2). HER4/ErbB4, like other family members, has four ectodomains, a single transmembrane domain and a cytoplasmic tail containing the active tyrosine kinase domain (3). By binding to neuregulins and/or EGF family ligands, ErbB4 forms either a homodimer or heterodimer with other ErbB family members, which results in receptor activation and signaling (3). ErbB4 is ubiquitously expressed with the highest expression occurring in brain and heart. The expression of ErbB4 in breast cancer, pediatric brain cancer and other types

of carcinomas has been reported suggesting that ErbB4 expression is involved in both normal tissue development and carcinogenesis (3).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing a fragment of human HER4/ErbB4 (Arg676-Val1308) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The substrate peptide was selected using our Tyrosine Kinase Substrate Screening Kit #7450. Phospho-Tyrosine mAb (P-Tyr-100) #9411 was used for detection. The quality of the biotinylated peptide was evaluated by reverse-phase HPLC and by mass spectrometry.

Purified HER4/ErbB4 kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. The specific activity of the HER4/ErbB4 kinase was determined using a radiometric assay [Fig.1]. Time course [Fig.2], kinase dose dependency [Fig.3] and substrate dose-dependency [Fig.4] assays were performed to verify HER4/ErbB4 activity using the HER4/ErbB4 substrate peptide provided in this kit. HER4/ErbB4 sensitivity to the inhibitor staurosporine was measured using the HER4/ErbB4 substrate peptide provided in this kit [Fig.5].

Background References:

- (1) Holbro, T. and Hynes, N.E. (2004) *Annu. Rev. Pharmacol. Toxicol.* 44, 195–217.
- (2) Roskoski Jr, R. (2004) *Biochem. Biophys. Res. Commun.* 319, 1–11.
- (3) Carpenter, G. (2003) *Exp. Cell Res.* 284, 66–77.

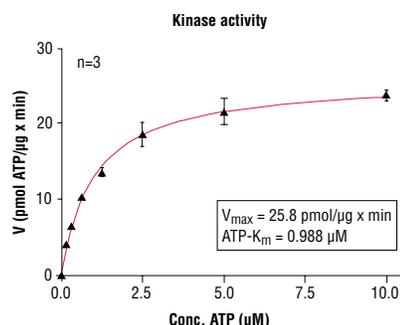


Figure 1. HER4/ErbB4 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM MgCl₂, 3 mM MnCl₂, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: PolyAEKY, 5 µg/50 µl, recombinant HER4/ErbB4: 50 ng/50 µl.

Storage: Antibodies are supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Peptides are supplied at 6 µM in 0.001% DMSO. Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at -80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Tyrosine Kinase Substrate Screening Kit #7450

HER4/ErbB4 Kinase #7724

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

PYK2 (Tyr402) Biotinylated Peptide #1315

Staurosporine #9953

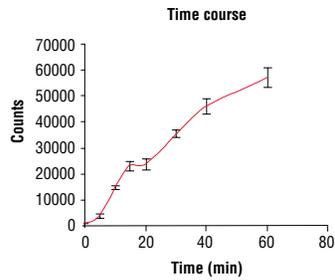


Figure 2. Time course of HER4/ErbB4 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of HER4/ErbB4 substrate peptide (#1315) by HER4/ErbB4 kinase. In a 50 µl reaction, 50 ng HER4/ErbB4 and 1.5 µM substrate peptide were used per reaction. (DELFLIA® is a registered trademark of PerkinElmer, Inc.)

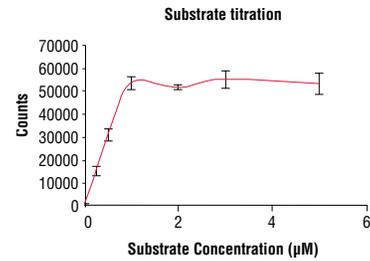


Figure 4. Peptide concentration dependence of HER4/ErbB4 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1315) by HER4/ErbB4 kinase. In a 50 µl reaction, 50 ng of HER4/ErbB4 and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFLIA® is a registered trademark of PerkinElmer, Inc.)

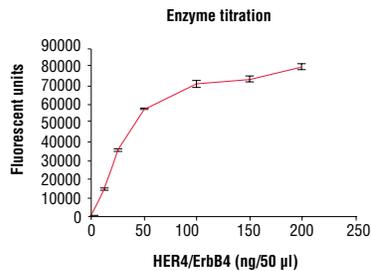


Figure 3. Dose dependence curve of HER4/ErbB4 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1315) by HER4/ErbB4 kinase. In a 50 µl reaction, increasing amounts of HER4/ErbB4 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFLIA® is a registered trademark of PerkinElmer, Inc.)

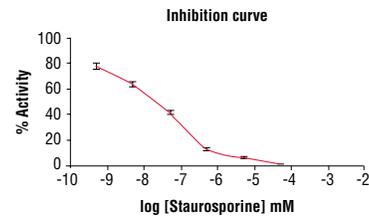


Figure 5. Staurosporine inhibition of HER4/ErbB4 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of HER4/ErbB4 substrate peptide (#1315) by HER4/ErbB4 kinase. In a 50 µl reaction, 50 ng HER4/ErbB4, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFLIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan® HER4/ErbB4 Kinase Assay Kit

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

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

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B Suggested Protocol for 100 Assays

1. Add 10 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH₂O to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=40 µM, [substrate]=3 µM).
2. Immediately 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 (1.25 M) to 2.5 ml of 4X HTScan® Tyrosine Kinase Buffer (240 mM HEPES pH 7.5, 20 mM MgCl₂, 20 mM MnCl₂, 12 µM Na₃VO₄) to make DTT/Kinase buffer.
5. Transfer 1.2 ml of DTT/Kinase buffer to each enzyme tube to make 4X reaction cocktail ([enzyme] = 4 ng/µL in 4X reaction cocktail).
6. Incubate 12.5 µl of the 4X reaction cocktail with 12.5 µl/well of prediluted compound of interest (usually around 10 µM) 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 MgCl₂
5 mM MnCl₂
3 µM Na₃VO₄
1.25 mM DTT
20 µM ATP
1.5 µM peptide
50 ng HER4/ErbB4 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 and 75 µl dH₂O/well to a 96-well streptavidin-coated plate 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), 1:1000 in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
13. Incubate at room temperature for 60 minutes.
14. *Wash three times with 200 µl/well PBS/T
15. For DELFLIA® or Colorimetric ELISA detection methods please use the following protocols.

DELFLIA® 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 DELFLIA® 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 DELFLIA®

DELFLIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFLIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFLIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFLIA® 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