Store at –80°C

#7058

HTScan® HER2/ErbB2 Kinase Assay Kit

☑ 100 assays
(96 Well Format)

Cell Signaling TECHNOLOGY®

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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, 1.25M)		80 µl
ATP (10 mM)	9804	1 ml
FLT3 (Tyr589) Biotinylated Peptide	1305	1.25 ml
HER2/ErbB2 Kinase (recombinant, human)	7382	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human HER2/ErbB2 kinase. It includes active HER2/ErbB2 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: NEY*FY*V

Molecular Weights: Peptide substrate, Biotin-FLT3 (Tyr589) peptide: 1,945 Daltons. GST-HER2/ErbB2 Kinase: 116 kDa.

Background: The ErbB2 (HER2) proto-oncogene encodes a transmembrane receptor-like glycoprotein of 185 kDa with intrinsic tyrosine kinase activity (1). ErbB2 does not have any known ligand. However, the kinase activity of ErbB2 can be activated without ligand if it is overexpressed and by heteromeric association with other members of the ErbB family (2). Amplification of the ErbB2 gene and overexpression of its product are detected in almost 40% of human breast cancers (3). Binding of the c-Cbl ubiquitin ligase to Tyr1112 of ErbB2 leads to poly-ubiquitination of ErbB2 and enhances its degradation (4). ErbB2 is one of the major targets for the treatment of breast cancer and other carcinomas. Direction of ErbB2 to the c-Cbl-regulated proteolytic pathway may have therapeutic potential.

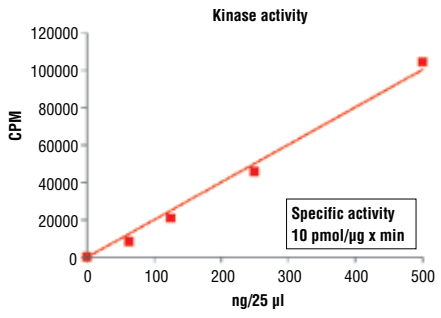

Figure 1. HER2/ErbB2 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 5 mM $MnCl_2$, 1 mM EGTA, 0.4 mM EDTA, 4 mM $MgCl_2$, 0.05 mM DTT, 50 µM ATP, Substrate: Poly(Glu-Tyr), 400 ng/µL, and recombinant HER2/ErbB2: variable.

Tyr877 of ErbB2 is homologous to Tyr416 of pp60c-Src, located in the kinase domain. Phosphorylation of this site may be involved in regulation of ErbB2 biological activity. Tyr1248 and Tyr1221/1222 are the major autophosphorylation sites in ErbB2. Phosphorylation of these sites couples ErbB2 to the Ras-Raf-MAP kinase signal transduction pathway (1,5).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing a fragment of human HER2/ErbB2 (Lys676-Val1255) (GenBank Accession No. NM_004448) 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 HER2/ErbB2 kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain. The specific activity of the HER2/ErbB2 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 HER2/ErbB2 activity using the HER2/ErbB2 substrate peptide provided in this kit. HER2/ErbB2 sensitivity to the inhibitor staurosporine was measured using the HER2/ErbB2 substrate peptide provided in this kit [Fig.5].

Background References:

(1) Muthuswamy, S.K. et al. (1999) *Mol. Cell. Biol.* 19, 6845–6857.

(2) Qian, X. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91, 1500–1504.

(3) Dittadi, R. and Gion, M. (2000) *J. Natl. Cancer Inst.* 92, 1443–1444.

(4) Klapper, L.N. et al. (2000) *Cancer Res.* 60, 3384–3388.

(5) Kwon, Y.K. et al. (1997) *J. Neurosci.* 17, 8293-8299.

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 7.5), 150 mM NaCl, 25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 7 mM reduced glutathione and 25% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Tyrosine Kinase Substrate Screening Kit #7450

HER2/ErbB2 Kinase #7382

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

FLT3 (Tyr589) Biotinylated Peptide #1305

Staurosporine #9953

HTScan® Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

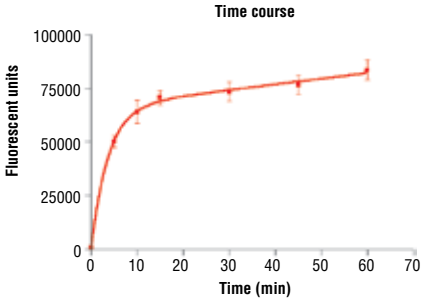

Figure 2. Time course of HER2/ErbB2 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of HER2/ErbB2 substrate peptide (#1305) by HER2/ErbB2 kinase. In a 50 µl reaction, 50 ng HER2/ErbB2 and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

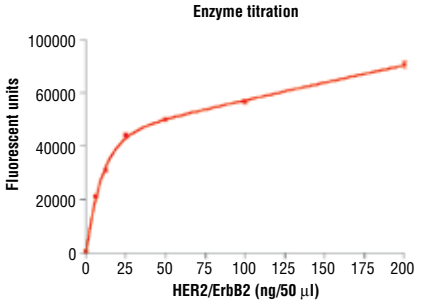

Figure 3. Dose dependence curve of HER2/ErbB2 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1305) by HER2/ErbB2 kinase. In a 50 µl reaction, increasing amounts of HER2/ErbB2 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

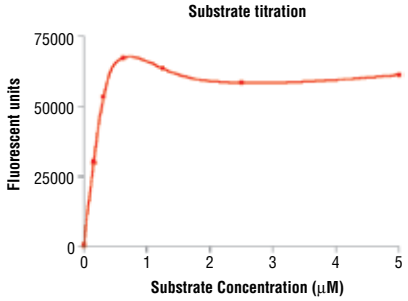

Figure 4. Peptide concentration dependence of HER2/ErbB2 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1305) by HER2/ErbB2 kinase. In a 50 µl reaction, 50 ng of HER2/ErbB2 and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

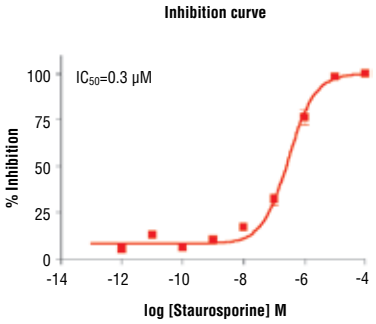

Figure 5. Staurosporine inhibition of HER2/ErbB2 kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of HER2/ErbB2 substrate peptide (#1305) by HER2/ErbB2 kinase. In a 50 µl reaction, 100 ng HER2/ErbB2, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan® HER2/ErbB2 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 dH_20 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 $MgCl_2$, 20 mM $MnCl_2$, 12 µM Na_3VO_4) 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 $MgCl_2$
5 mM $MnCl_2$
3 µM Na_3VO_4
1.25 mM DTT
20 µM ATP
1.5 µM peptide
50 ng HER2/ErbB2 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 dH_2O/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 DELFIA® or Colorimetric ELISA detection methods please use the following protocols.

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