Store at –80°C

#7746

HTScan® IGF-1 Receptor 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
ATP (10 mM)	9804	1 ml
IRS-1 (Tyr891) Biotinylated Peptide	1320	1.25 ml
IGF-I Receptor Kinase (recombinant, human)	7745	5 µg

Description: The kit provides a means of performing kinase activity assays with recombinant human IGF-IR kinase. It includes active IGF-IR 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: GEY*VN

Molecular Weights: Peptide substrate, Biotin-peptide: 2,005 Daltons. GST-IGF-IR Kinase: 77 kDa.

Background: Type I insulin-like growth factor receptor (IGF-IR), a transmembrane receptor tyrosine kinase, is widely expressed in many cell types within fetal and postnatal tissues, and in many cell lines (1-3). Upon binding to its ligands, IGF-I and IGF-II, receptor autophosphorylation occurs. The triple tyrosine cluster within the kinase domain (Tyr1131, Tyr1135 and Tyr1136) is the earliest major site of autophosphorylation (4). Phosphorylation of these three tyrosine residues is necessary for kinase activation (5,6).

Insulin receptors (IRs) share significant similarity with IGF-I receptors in both structure and function. There is also an equivalent triple tyrosine cluster within the activation loop of the kinase domain (Tyr1146, Tyr1150 and Tyr1151). Tyrosine autophosphorylation of insulin receptor is one of the earliest cellular responses to insulin stimulation (7). Autophosphorylation begins with phosphorylation of Tyr1146 and either Tyr1150 or Tyr1151. Full kinase activation requires the triple tyrosine phosphorylation (8).

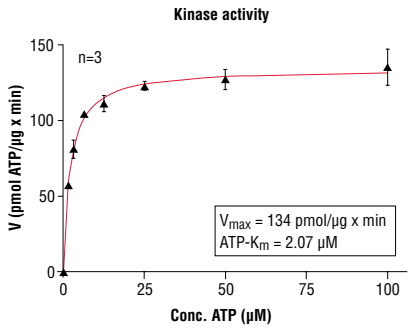

Figure 1. IGF-IR kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20.000, Substrate: PolyEY, 2 µg/50 µl, recombinant IGF-IR: 20 ng/50 µl.

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing a fragment of human IGF-IR (Met954-Cys1367) (GenBank accession No. NM_000875) 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 IGF-IR kinase was quality controlled for purity by SDS-PAGE followed by Coomassie stain and Western blot. The specific activity of the IGF-IR 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 IGF-IR activity using the IGF-IR substrate peptide provided in this kit. IGF-IR sensitivity to the inhibitor staurosporine was measured using the IGF-IR substrate peptide provided in this kit [Fig.5].

Background References:

(1) Adams, T.E. et al. (2000) *Cell. Mol. Life Sci.* 57, 1050–1093.

(2) Baserga, R. et al. (2000) *Oncogene* 19, 5574–5581.

(3) Scheidegger, K.J. et al. (2000) *J. Biol. Chem.* 275, 38921–38928.

(4) Hernandez-Sanchez, C. et al. (1995) J. Biol. Chem. 270, 29176-29181.

(5) Lopaczynski, W. et al. (2000) *Biochem. Biophys. Res. Commun.* 279, 955–960.

(6) Baserga, R. et al. (1999) *Exp. Cell Res.* 253, 1–6.

(7) White, M.F. et al. (1985) *J. Biol. Chem.* 260, 9470–9478.

(8) White, M.F. et al. (1988) *J. Biol. Chem.* 263, 2969–2980.

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

IGF-I Receptor Kinase #7745

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

IRS-1 (Tyr891) Biotinylated Peptide #1320

Staurosporine #9953

HTScan® Profiling Kit (Tyrosine Kinase Set I) #7405

HTScan® Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

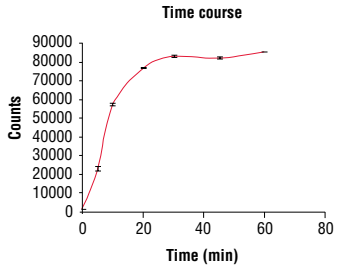

Figure 2. Time course of IGF-IR kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of IGF-IR substrate peptide (#1320) by IGF-IR kinase. In a 50 µl reaction, 50 ng IGF-IR and 1.5 µM substrate peptide were used per reaction. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

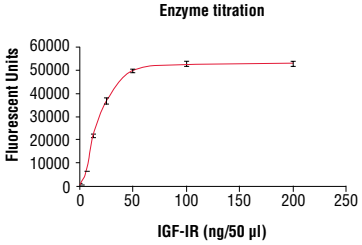

Figure 3. Dose dependence curve of IGF-IR kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1320) by IGF-IR kinase. In a 50 µl reaction, increasing amounts of IGF-IR and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

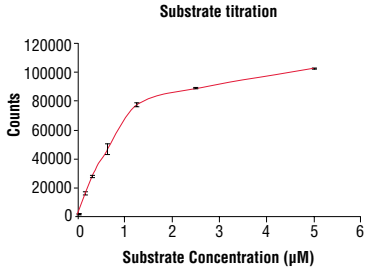

Figure 4. Peptide concentration dependence of IGF-IR kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1320) by IGF-IR kinase. In a 50 µl reaction, 50 ng of IGF-IR and increasing concentrations of substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

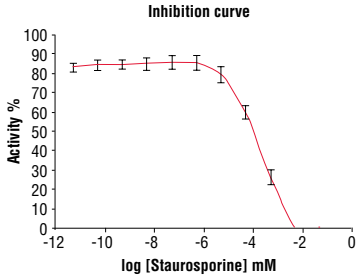

Figure 5. Staurosporine inhibition of IGF-IR kinase activity: DELFIA® data generated using Phospho-Tyrosine mAb (P-Tyr-100) #9411 to detect phosphorylation of IGF-IR substrate peptide (#1320) by IGF-IR kinase. In a 50 µl reaction, 50 ng IGF-IR, 1.5 µM substrate peptide, 20 µM ATP and increasing amounts of staurosporine were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan® IGF-1 Receptor 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 IGF-1 Receptor 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