#7405 *Various Storage Conditions

HTScan™ Profiling Kit (Tyrosine Kinase Set I)

☑ 8 Kinases
12 Reactions/Kinase

Box 1: Store at -70°C or -80°C
Box 2: Store at -20°C

new 12/04

Cell Signaling TECHNOLOGY®

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orders@cellsignal.com

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Products Included	Product #	Kit Quantity
ATP (10 mM)	9804	1 ml
DTT (1000x, 1.25 M)		80 µl
Phospho-Tyrosine Mouse mAb (P-Tyr-100)	9411	30 µl
HTScan™ Tyrosine Kinase Buffer (4x)	9805	15 ml
8 Tyrosine Kinases		200 U/each
Prearrayed biotinylated substrates		96-well plate

Description: The kit provides a means of characterizing the effect of a compound or panel of compounds on the included active human receptor tyrosine kinases.

The tyrosine kinases in the kit are supplied as GST fusion proteins. Biotinylated substrate peptides validated for each kinase are supplied at 6 µM, and a phosphotyrosine monoclonal antibody for detection of the phosphorylated form of the substrate peptide is also included in the kit. The kit enables rapid characterization of the effect of a single compound at multiple concentrations or a panel of compounds toward the following RTKs: PDGFRβ, EGFR, FGFR3, VEGFR-2, FLT3, IGF1R, MET and EPHB3, each representing an RTK subgroup.

Background: Tyrosine kinases have been validated as targets for therapeutic intervention in many disease areas. The human genome codes for nearly one hundred tyrosine kinases, which can be categorized into receptor tyrosine kinases (RTKs) and cytosolic tyrosine kinases. RTKs are currently a major focus for therapeutic drug target screening in the pharmaceutical industry. HTScan™ Profiling Kit (Tyrosine Kinase Set 1) from Cell Signaling Technology (CST) is an antibody-based kinase activity assay kit.

Each kinase is paired with a validated biotinylated substrate peptide, provided in 96-well format. Optimal assay conditions have been identified for each substrate peptide using a phosphotyrosine-specific antibody to achieve signal-to noise ratios over 25.

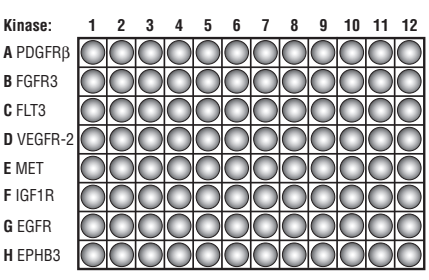
Unit Definition: 10 Units is defined as the amount of kinase required to maximally phosphorylate 75 pmol of biotinylated substrate peptide in 30 minutes at 25°C in a total reaction volume of 50 µl quantified by DELFIA® to achieve signal-to-noise ratios over 25. See vial label for lot-specific information.

Source/Purification: Each GST-Kinase fusion protein in this kit was produced using a baculovirus-expressed cDNA fragment with the target human tyrosine kinase domain amino-terminally fused to a GST-HIS_6-Thrombin cleavage site. Proteins were then purified by one-step affinity chromatography using glutathione-agarose.

The detection antibody, Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411, was derived from mice immunized with phospho-tyrosine-containing peptides (KLH-coupled).

Quality Control: Each substrate peptide was selected using Tyrosine Kinase Substrates Screening Kit #7450. The quality of the biotinylated peptides was evaluated by reversed-phase HPLC and by mass spectrometry.

The purity of each RTK was characterized using SDS-PAGE, and assay conditions for phosphorylation of the included substrates were optimized using DELFIA® (for details, visit www.cellsignal.com/drug2.ASP) (DELFIA® is a registered trademark of PerkinElmer, Inc.).



Assay plate (96 well format): Peptide substrates for the indicated eight RTKs have been arrayed in 96-well format.

Storage: Antibodies are supplied in in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Do not aliquot the antibodies. Store at –20°C. Peptides are supplied at 6 µM in 0.001% DMSO or a DMSO and carbonate buffer solution (15 mM Na_2CO_3, 35 mM $NaHCO_3$, 0.05% NaN_3). Store at –20°C. 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

Serine/Threonine Kinase Substrate Screening Kit #7400

HTScan™ EGFR Kinase Assay Kit #7410

HTScan™ EPHB3 Kinase Assay Kit #7716

HTScan™ IGFR Kinase Assay Kit #7746

HTScan™ MET Kinase Assay Kit #7440

HTScan™ VEGFR-2 Kinase Assay Kit #7788

HTScan™ FLT3 Kinase Assay Kit #7743

FLT3 (Tyr589) Biotynilated Peptide #1305

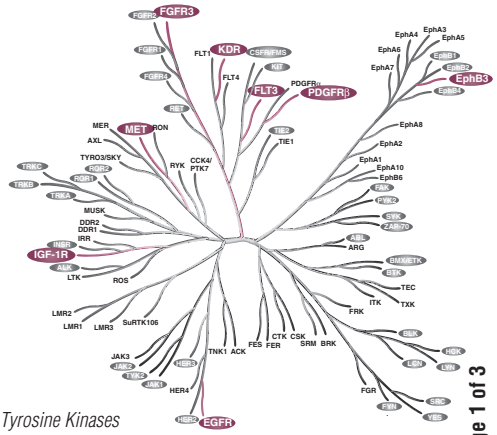
Gastrin Precursor (Tyr87) Biotinylated Peptide #1310

PYK2 (Tyr402) Biotinylated Peptide #1315

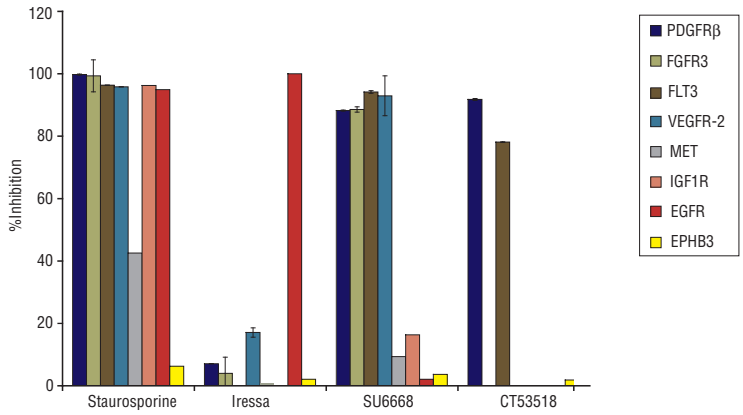
IRS1 (Tyr891) Biotinylated Peptide #1320

PTP1B (Tyr66) Biotinylated Peptide #1325

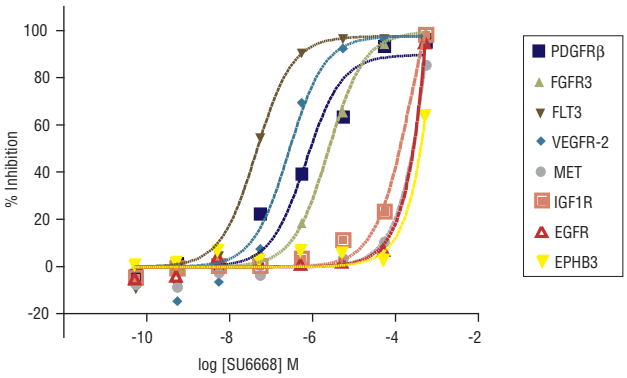
BTK (Tyr223) Biotinylated Peptide #1330



Tyrosine Kinases



Compound panel (5 µM each compound): DELFIA® data were generated using HTScan™ Profiling Kit (Tyrosine Kinase Set I) to detect the inhibition of various RTKs by a panel of compounds (Staurosporine, Iressa, SU-6668 and CT53518). In a 50 µl reaction, 10 Units of each RTK, 1.5 µM substrate peptide, 20 µM ATP and 5 µM inhibitor were used per reaction well (in duplicate) at 25°C for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)



Kinase	IC_{50}(µM)
PDGFRβ	0.68
FGFR3	2.4
FLT3	0.043
VEGFR-2	0.26
MET	250
IGF1R	123
EGFR	240
EPHB3	407

Dose response (SU-6668): DELFIA® data were generated using HTScan™ Profiling Kit (Tyrosine Kinase Set I) to detect the inhibition profile of SU-6668 toward various RTKs. In a 50 µl reaction, 10 Units of each GST-RTK, 1.5 µM substrate peptide, 20 µM ATP and various concentrations of SU6668 were used per reaction well at 25°C for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for HTScan™ Profiling Kit (Tyrosine Kinase Set 1)

Suggested Kinase Assay Conditions:

■ Combine 12.5 µl of 6 µM peptide substrate and 12.5 µl H_2O with compound of interest. Add 2x kinase reaction cocktail (see below). Incubate at 25°C for 30 minutes.

Peptide substrates are supplied in H_2O plus 0.001 % DMSO.

Note: Lot-specific information for each kinase is provided on the enzyme vial.

Additional Solutions and Reagents (Not included)

■ Wash Buffer: 1X PBS, 0.05% Tween-20 (PBS/T)

■ Bovine Serum Albumin (BSA)

■ Stop Buffer: 50 mM EDTA pH 8

■ DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)

■ DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)

■ DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFIA® is a registered trademark of PerkinElmer Life Sciences

Suggested Protocol

1. Transfer enzymes from -80°C immediately to ice. Allow enzyme to thaw on ice.
2. **Microcentrifuge briefly at 4°C to bring all liquid to the bottom of the vial. Return immediately to ice.**
3. Transfer 12.5 µl of substrate peptides to plate wells containing 12.5 µl of diluted compounds or H_20.
4. Set up 2x Reaction cocktails (Mock/No Kinase and Plus Kinase) in tubes on ice.

   2.5 ml 4X HTScan™ Tyrosine Kinase Buffer (240 mM HEPES pH 7.5, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 µM Na_3VO_4)

   20 µl ATP (10 mM)

   10 µl DTT (1.25 M)

   Bring to 5 ml with H_20. Mix gently by inversion

   Transfer each enzyme to 0.5 ml 2X reaction cocktail. Final enzyme concentration should be 10 Units/25 µl in 2X reaction cocktail. Immediately proceed to step 5.
5. Add 25 µl 2x kinase reaction cocktail (containing kinase) to 25 µl diluted peptide in each well. Mix by gentle agitation.

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

10 Units Kinase

6. Incubate reaction plate at 25°C for 30 minutes.
7. Add 50 µl Stop Buffer (50 mM EDTA, pH 8) to each well and mix by pipetting.
8. Transfer 25 µl of each reaction and 75 µl H_2O/well to a 96-well streptavidin-coated plate and incubate at room temperature for 60 minutes.
9. *Wash three times with 200 µl/well PBS/T.
10. Dilute primary antibody, Phospho-Tyrosine Monoclonal Antibody (P-Tyr-100) #9411, 1:1000 in PBS/T with 1% BSA. Add 100 µl primary antibody per well.
11. Mix and incubate at room temperature with rocking for 60 minutes.
12. *Wash three times with 200 µl/well PBS/T.
13. Dilute Europium labeled anti-mouse IgG 1:500 in PBS/T with 1% BSA. Add 100 µl diluted antibody/well.
14. Mix and incubate at room temperature for 30 minutes.
15. *Wash five times with 200 µl/well PBS/T.
16. Add 100 µl/well DELFIA® Enhancement Solution.
17. Incubate at room temperature for 5 minutes.
18. Detect 615 nm fluorescence emission with 340 nm excitation using appropriate Time-Resolved Plate Reader (400 µS delay).

***IMPORTANT: 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