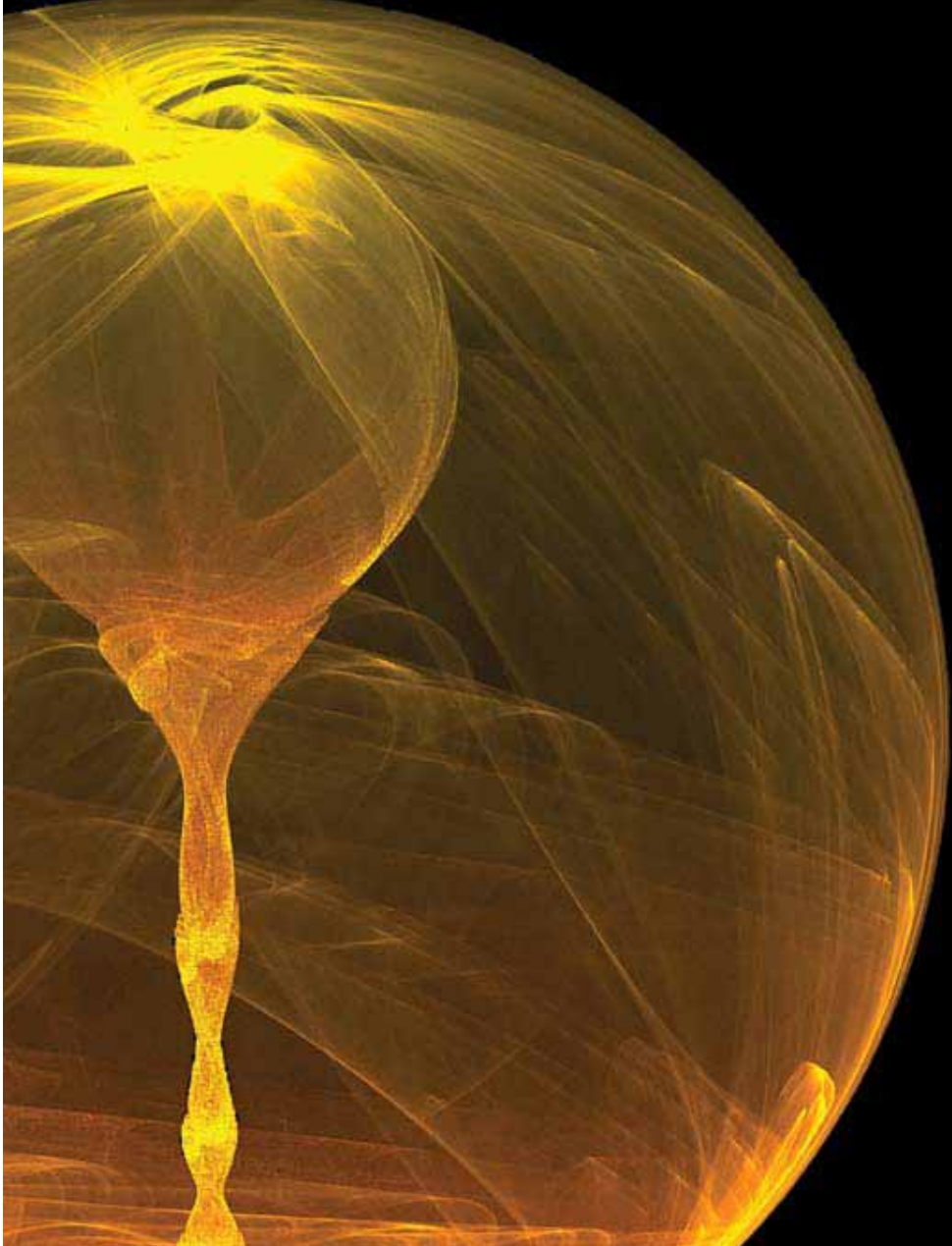
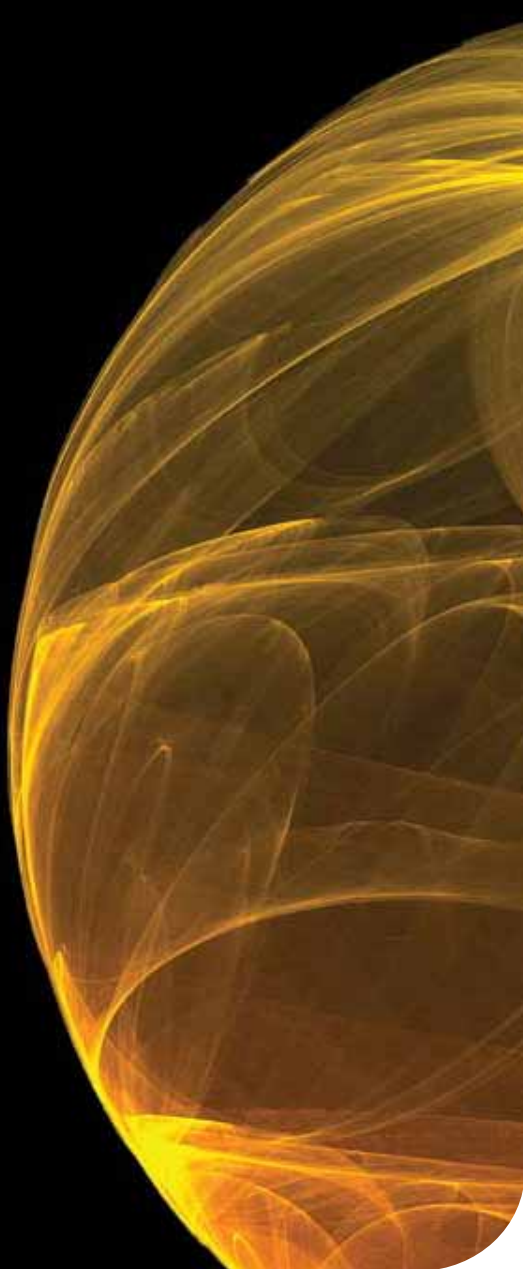


Novagen®

WideScreen™

RTK Assays

for the Luminex® xMAP® Technology platform



# WideScreen™

## RTK Assays

using Luminex® xMAP® Technology

Membrane-bound Receptor Tyrosine Kinases (RTKs) act as gatekeepers for many signaling pathways. RTKs dimerize when bound to their ligand, resulting in the activation of intracellular autophosphorylation domains. Typically, this signal is transmitted to intracellular downstream targets via phosphotyrosine-SH2 domain interactions.

The phosphorylation status of RTKs is of interest to cell biologists, researchers investigating disease states, and drug development scientists. A number of drugs have been developed which specifically target RTKs and tools are needed to determine the effects of these novel therapeutic compounds and proteins.

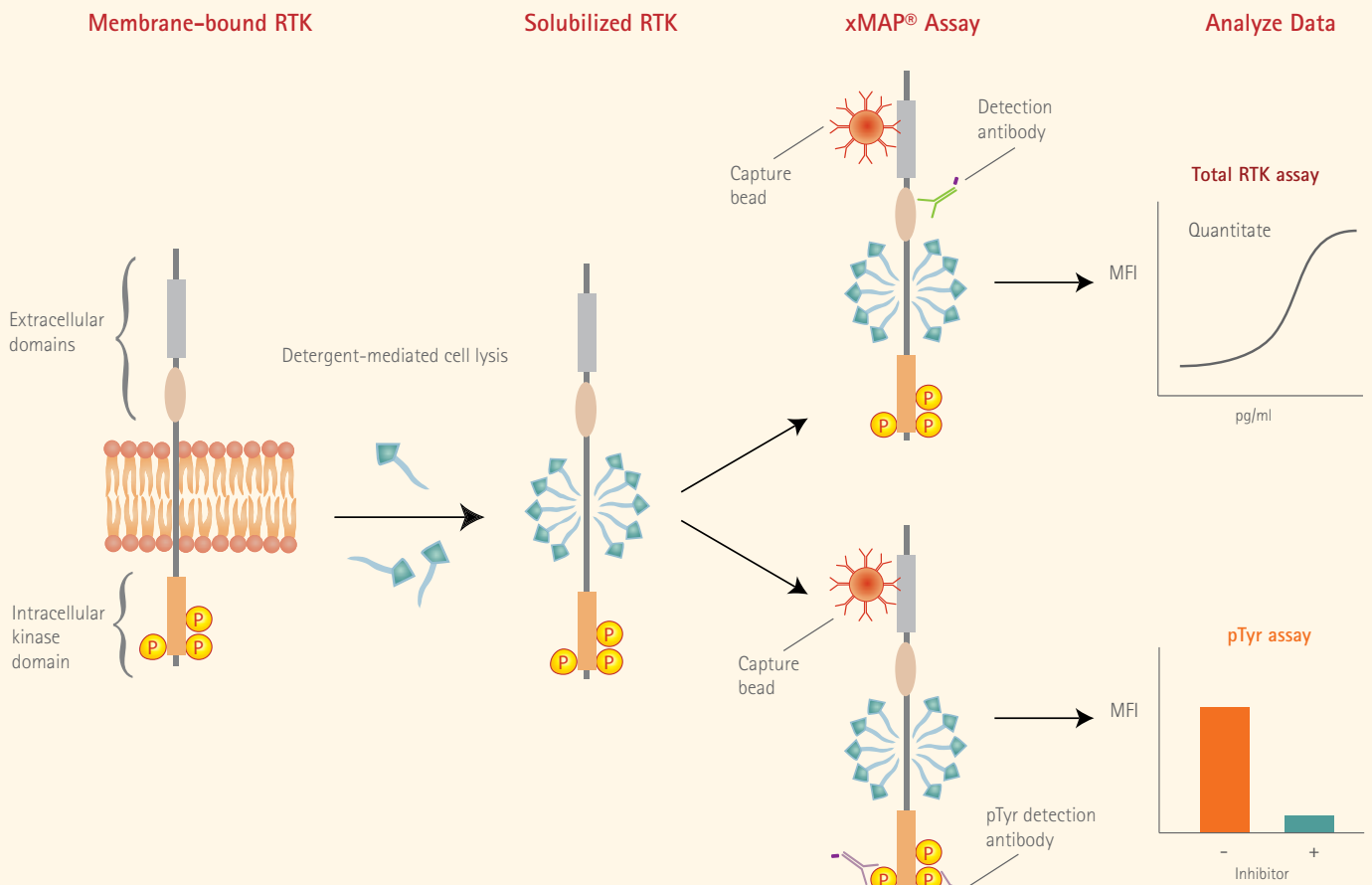


WideScreen Group,  
Darmstadt, Germany

The Novagen® WideScreen™ Group, an international interdisciplinary team of assay development scientists, was assembled to introduce the next generation of multiplex immunoassays. After extensive R&D, the first wave of unique assay panels created by the Group are available for the bead-based Luminex® xMAP® Technology platform.

# WideScreen™ RTK Assays Principle

The Novagen® WideScreen™ Group has developed bead-based assays to simultaneously determine the tyrosine phosphorylation status of several key Receptor Tyrosine Kinases (RTKs). The WideScreen RTK Assays consist of a series of phosphotyrosine-specific RTK assays and companion total RTK assays. The phosphotyrosine assays utilize specific capture antibodies and a broad-spectrum phosphotyrosine detection antibody. The total RTK assays, which include standards, allow the signals from the phosphotyrosine assays to be compared to the amount of RTK in the sample.

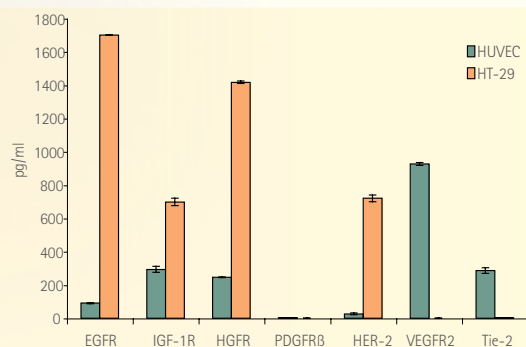
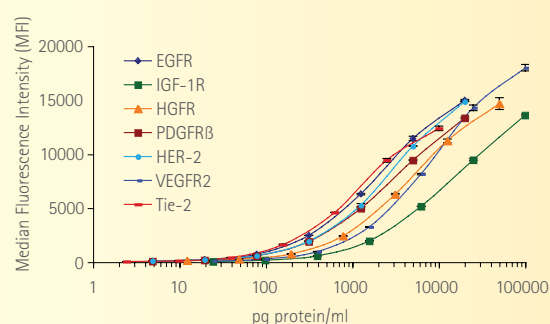


# WideScreen™ RTK Assays

The WideScreen™ RTK Assays can be applied to study cell signaling as well as the effect of inhibitors on a wide range of RTKs. The RTK Total Assays enable the quantitation of RTKs for expression profiling, confirming knock-down experiments, and for the normalization of protein levels during inhibitor profiling. The RTK pTyr Assays can be used in secondary screens, to determine the off-target effects of compounds, and have general applications in the analysis of RTK phosphorylation.

## WideScreen™ RTK Total Assay Standard Curves

The RTK Total Standards Mix, 7-Plex is used to create standard curves to determine the amount of total RTK levels. Recombinant standards are provided for each analyte. Typical standard curves developed with the WideScreen RTK Total Assay Complete Kit are shown.

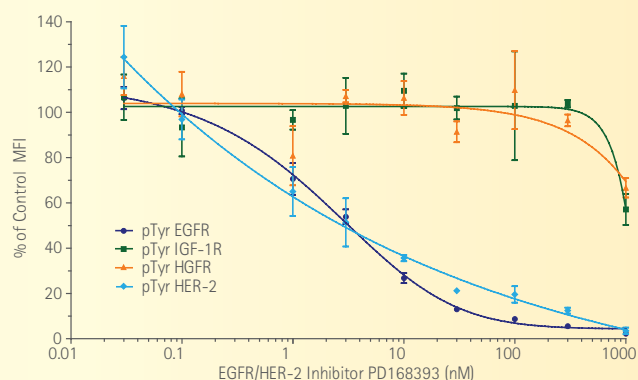


## RTK Expression in Different Cell Lines

HUVEC and HT-29 cell lysates (10 µg total protein) were analyzed with the WideScreen RTK Total Assay Complete Kit to determine expression levels and concentrations of EGFR, IGF-1R, HGFR, PDGFRB, HER-2, VEGFR2, and Tie-2.

## Inhibitor Profiling

HT-29 cells were cultured in 96-well plates. Starved cells were incubated with increasing amounts of the EGFR/HER-2 inhibitor PD168393 (Calbiochem Cat. No. 513033) for 1 h and stimulated with a cocktail of growth factors for 10 min. Levels of phosphorylated RTK in cell lysates were tested with the WideScreen RTK pTyr Assay Complete Kit. Levels of phosphorylated protein are shown as percent of positive control (vehicle-treated HT-29 cells). The data show that PD168393 is specific for EGFR/HER-2 and does not inhibit the phosphorylation of IGF-1R or HGFR at sub-micromolar concentrations. HT-29 cells do not express significant amounts of VEGFR2, Tie-2, or PDGFRB.



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[www.novagen.com/WideScreen](http://www.novagen.com/WideScreen)

## WideScreen™ RTK Total and pTyr Assays

the most comprehensive panels for the analysis of RTK levels and activity.

### WideScreen RTK Total Assay

Expression profiling  
Confirmation of knock-downs  
Biomarker quantitation

### WideScreen RTK pTyr Assay

Inhibitor profiling  
Pathway analysis  
Compound screening

## Flexible Configuration

The WideScreen™ RTK Assays are designed to be flexible.

The assays are available as Complete Kits, which contain all lysis and wash buffers, premixed beads and detection cocktails needed to run 96 assays. The Cell Extraction and WideScreen Reagent Kits are also available separately, allowing greater cell culture and processing options.

- The two premixed WideScreen RTK Assays are for the separate detection of total and pTyr-RTKs
- Individual bead kits allow the creation of customer-defined multiplexes\*

### WideScreen RTK Total Assay Complete Kit

#### RTK Total 7-Plex

Premixed capture beads, detection antibodies and standards:

Total EGFR  
Total IGF-1R  
Total HGFR  
Total PDGFRβ  
Total HER-2  
Total VEGFR2  
Total Tie-2

Cell Extraction Kit<sup>1</sup>

WideScreen Reagent Kit<sup>2</sup>

### WideScreen RTK pTyr Assay Complete Kit

#### RTK pTyr 7-Plex

Premixed capture beads and detection antibody:

pTyr EGFR  
pTyr IGF-1R  
pTyr HGFR  
pTyr PDGFRβ  
pTyr HER-2  
pTyr VEGFR2  
pTyr Tie-2

Cell Extraction Kit

WideScreen Reagent Kit

### WideScreen RTK User-defined assay\*

Choice of:

RTK  
Total  
7-Plex

RTK  
pTyr  
7-Plex

Individual  
Bead Kits\*

Cell Extraction Kit

WideScreen Reagent Kit

Cell Extraction Kit includes Benzonase® Nuclease to reduce sample viscosity and enhance assay performance.

\*Total- and pTyr-assays for the same analyte *cannot be multiplexed together*.

<sup>1</sup> Cell Extraction Kit includes: Extraction Reagent, Benzonase® Nuclease, Phosphatase Inhibitor Cocktail Set V, and Protease Inhibitor Cocktail Set III.

<sup>2</sup> WideScreen Reagent Kit includes: 96-Well Filter Plate and Sealer, Streptavidin-PE Concentrate, Assay Diluent, and Wash Buffer.

Product	Components	Size	Cat. No.	
Total	WideScreen™ RTK Total Assay Complete Kit	Cell Extraction Kit, WideScreen Reagent Kit, RTK Total 7-Plex. For the detection of total EGFR, IGF-1R, HGFR, PDGFRβ, HER-2, VEGFR2 and Tie-2	96 tests	71942-3
	RTK Total 7-Plex	RTK Capture Beads Premix, 7-Plex, RTK Total Detection Antibody Premix, 7-Plex, RTK Total Standards Mix, 7-Plex. For the detection of total EGFR, IGF-1R, HGFR, PDGFRβ, HER-2, VEGFR2 and Tie-2	100 tests	71924-3
	EGFR Total Bead Kit	EGFR Capture Beads, EGFR Total Detection Antibody, EGFR Total Standard	100 tests	71928-3
	IGF-1R Total Bead Kit	IGF-1R Capture Beads, IGF-1R Total Detection Antibody, IGF-1R Total Standard	100 tests	71929-3
	HGFR Total Bead Kit	HGFR Capture Beads, HGFR Total Detection Antibody, HGFR Total Standard	100 tests	71930-3
	PDGFRβ Total Bead Kit	PDGFRβ Capture Beads, PDGFRβ Total Detection Antibody, PDGFRβ Total Standard	100 tests	71931-3
	HER-2 Total Bead Kit	HER-2 Capture Beads, HER-2 Total Detection Antibody, HER-2 Total Standard	100 tests	71932-3
	VEGFR2 Total Bead Kit	VEGFR2 Capture Beads, VEGFR2 Total Detection Antibody, VEGFR2 Total Standard	100 tests	71933-3
	Tie-2 Total Bead Kit	Tie-2 Capture Beads, Tie-2 Total Detection Antibody, Tie-2 Total Standard	100 tests	71934-3
pTyr	WideScreen™ RTK pTyr Assay Complete Kit	Cell Extraction Kit, WideScreen Reagent Kit, RTK pTyr 7-Plex. For the detection of pTyr EGFR, pTyr IGF-1R, pTyr HGFR, pTyr PDGFRβ, pTyr Her-2, pTyr VEGFR2 and pTyr Tie-2	96 tests	71943-3
	RTK pTyr 7-Plex	RTK Capture Beads Premix, 7-Plex, pTyr Detection Antibody. For the detection of pTyr EGFR, pTyr IGF-1R, pTyr HGFR, pTyr PDGFRβ, pTyr Her-2, pTyr VEGFR2 and pTyr Tie-2	100 tests	71925-3
	EGFR pTyr Bead Kit	EGFR Capture Beads, pTyr Detection Antibody	100 tests	71935-3
	IGF-1R pTyr Bead Kit	IGF-1R Capture Beads, pTyr Detection Antibody	100 tests	71936-3
	HGFR pTyr Bead Kit	HGFR Capture Beads, pTyr Detection Antibody	100 tests	71937-3
	PDGFRβ pTyr Bead Kit	PDGFRβ Capture Beads, pTyr Detection Antibody	100 tests	71938-3
	HER-2 pTyr Bead Kit	HER-2 Capture Beads, pTyr Detection Antibody	100 tests	71939-3
	VEGFR2 pTyr Bead Kit	VEGFR2 Capture Beads, pTyr Detection Antibody	100 tests	71940-3
	Tie-2 pTyr Bead Kit	Tie-2 Capture Beads, pTyr Detection Antibody	100 tests	71941-3
Cell Extraction Kit	Extraction Reagent, Phosphatase Inhibitor Cocktail Set V, Benzomase® Nuclease HC, Purity > 99%, Protease Inhibitor Cocktail Set III	1 kit	71926-3	
WideScreen™ Reagent Kit	96-Well Filter Plate and Sealer, Streptavidin-PE Concentrate, 5X Assay Diluent, 10X Wash Buffer	1 kit	71783-3	

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