

TRANSCRIPTION FACTOR ANALYSIS

STAT DuoSet® IC ELISA Development Systems

Signal transducer and activator of transcription (STAT) proteins are transcription factors activated in response to cytokine, growth factor, or hormone receptor signaling. Janus kinases (JAKs) phosphorylate STAT proteins and induce dimerization. Homo- or heterodimers translocate to the nucleus where they bind to DNA and activate transcription.

R&D Systems has developed colorimetric assays to measure activated transcription factors by using a modified ELISA format that is fast, simple, sensitive, and can be easily adapted to high-throughput analysis. Typical results show excellent correlation with traditional EMSA and Western blot analyses (Figure 1). The Active STAT DuoSet ELISA may be used with biotinylated anti-STAT antibodies to detect heterodimer formation (Figure 2) and/or total STAT protein (Figure 3).

DUOSET IC ELISAs AND ACTIVITY ASSAYS	SPECIES	CATALOG #
Active STAT1 p91	human/mouse	DYC1490
Active STAT4	human	DYC1574
Active p53	human/mouse	DYC1355
Total p53	human	DYC1043
	mouse	DYC1746

ANTIBODY	SPECIES	APPLICATIONS	CATALOG #
HIF-1 α	human/mouse	WB	AB1536
STAT1 p91 aa 702-739	human/mouse	WB, IP	PAF-ST1
STAT2 aa 679-851	human	WB, IP	PAF-ST2
STAT3 aa 747-761	human/mouse	WB, IP	PA-ST3
STAT4 aa 675-749	human/mouse	WB, IP	PAF-ST4
STAT4 aa 730-749	human/mouse	WB, IP	PA-ST4
STAT5a aa 775-794	human/mouse	WB, IP	PA-ST5A
STAT5b aa 777-787	human/mouse	WB, IP	AF1584
STAT5b aa 769-782	human/mouse	WB, IP	PA-ST5B
STAT6 aa 818-837	human/mouse	WB, IP	PA-ST6
STAT1 p91 aa 739-933, Biotin	human/mouse	Total STAT1 p91 Analysis	BAF1490
STAT2 aa 679-851, Biotin	human	WB, Total STAT2 Analysis, STAT1 p91/STAT2 Heterodimer Analysis	BAF1666
STAT4 aa 675-749, Biotin	human, mouse	Total STAT4 Analysis	BAF1574

Key: WB Western blot IP Immunoprecipitation

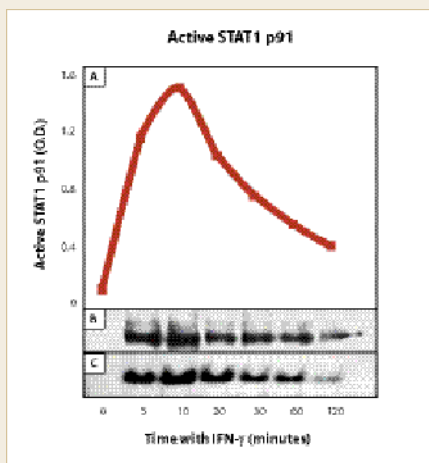


Figure 1. Human U937 cells were treated with 100 ng/mL R&D Systems' recombinant human IFN- γ (Catalog # 285-IF) for the indicated times. **A.** Nuclear extracts were prepared and assayed at 5 μ g/well using R&D Systems' Active Human/Mouse STAT1 p91 DuoSet IC ELISA (Catalog # DYC1490). **B.** The same extracts were analyzed by Western blot using R&D Systems' goat anti-human/mouse STAT1 p91 affinity purified polyclonal antibody (Catalog # PAF-ST1). **C.** EMSA was performed using 10 μ g nuclear extracts incubated with 30 fmol biotinylated double-stranded oligonucleotide and electrophoresed on a 5% non-denaturing polyacrylamide gel.

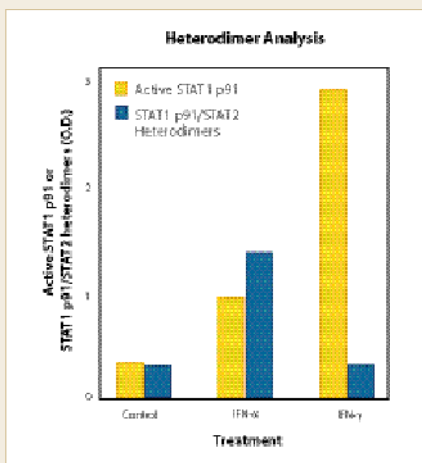


Figure 2. Human HeLa cells were treated with R&D Systems' recombinant human IFN- α 2a (Catalog # 11100-1) at 500 U/mL or recombinant human IFN- γ (Catalog # 285-IF) at 100 ng/mL for 20 minutes. Nuclear extracts were prepared and assayed at 10 μ g/well for active STAT1 p91 or STAT1 p91/STAT2 heterodimers using R&D Systems' Human/Mouse Active STAT1 p91 DuoSet IC ELISA (Catalog # DYC1490) and biotinylated goat anti-human STAT2 affinity purified polyclonal antibody (Catalog # BAF1666).

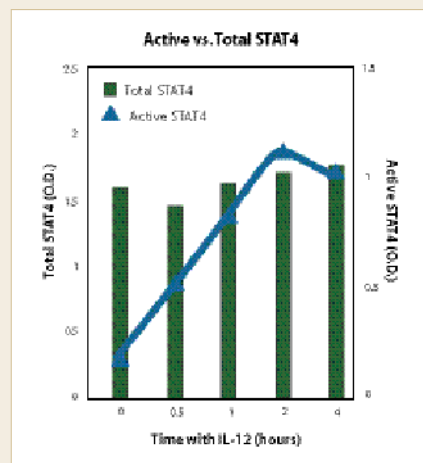


Figure 3. Human NK92 cells were treated with 100 ng/mL R&D Systems' recombinant human IL-12 (Catalog # 219-IL) for the indicated times. Nuclear extracts were prepared and assayed at 5 μ g/well for active and total STAT4 using R&D Systems' Human Active STAT4 DuoSet IC ELISA (Catalog # DYC1574) and biotinylated goat anti-human/mouse STAT4 affinity purified polyclonal antibody (Catalog # BAF1574).

PHOSPHO-PROTEIN ANALYSIS

Phospho-ERK2 (T185/Y187) DuoSet® IC ELISA Development System

The Raf-MEK-ERK signal transduction cascade functions in cellular proliferation, differentiation, and survival. Its inappropriate activation is a common occurrence in human cancers. Raf-activated MEK1 and MEK2 activate ERK1 and ERK2 by dual phosphorylation, at T202/Y204 for human ERK1 and T185/Y187 for human ERK2. While ERK1 and ERK2 are highly homologous and each is expressed ubiquitously, some differences in their activities have been noted, including their roles in mesoderm differentiation and MEK scaffolding. Both ERKs are proline-directed Ser/Thr kinases, phosphorylating an array of transcription factors and also regulating transcription indirectly by activating kinases from the RSK and MSK families.

The phospho-ERK2 (T185/Y187) DuoSet IC ELISA (Cat. # DYC1483; Figures 1 & 2) is one of a growing number of R&D Systems' DuoSet ELISA Development Systems for the measurement of phosphorylated intracellular signaling proteins. DuoSet IC ELISAs provide a fast, economical, and quantitative alternative to Western blots that can be easily adapted to high-throughput analysis.

DUOSET IC ELISAs	SPECIES	CATALOG #
phospho-ERK1 (T202/Y204)	human/mouse/rat	DYC1825
phospho-ERK1/ERK2	human/mouse/rat	DYC1018
phospho-ERK2 (T185/Y187)	human/mouse/rat	DYC1483
phospho-JNK (<i>pan-specific</i>)	human/mouse/rat	DYC1387
Total p21/CIP1/CDKN1A	human	DYC1047
phospho-p38 α (T180/Y182)	human/mouse/rat	DYC869
Total p38 α	human/mouse/rat	DYC8691
phospho-p38 γ (T183/Y185)	human/mouse	DYC1664
Total p38 γ	human/mouse/rat	DYC1347
phospho-p70 S6 Kinase (T389)	human	DYC896
phospho-RSK1 (S380)	human/mouse/rat	DYC892

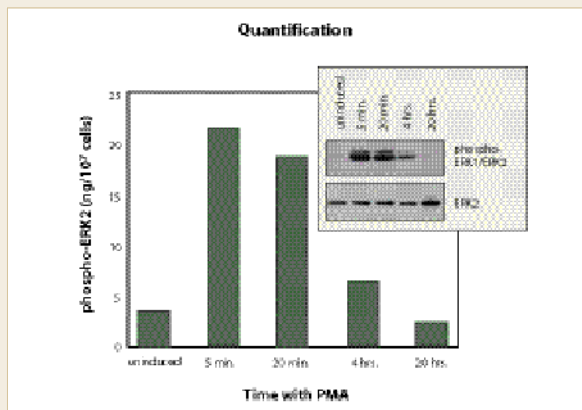


Figure 1. HeLa cells were treated with 200 nM PMA for the indicated times. Cells were lysed and either quantified using R&D Systems' phospho-ERK2 (T185/Y187) DuoSet IC ELISA (Catalog # DYC1483) or immunoblotted using R&D Systems' rabbit anti-phospho-ERK1/ERK2 (Catalog # AF1018; lower band corresponds to phospho-ERK2) or rabbit anti-ERK2 (Catalog # AF1230) affinity purified polyclonal antibodies.

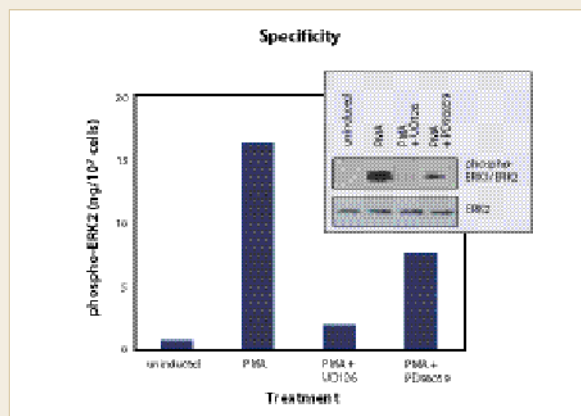


Figure 2. HeLa cells were incubated with no additions or with 200 nM PMA for 20 minutes, in the presence or absence of either 50 μ M U0126 or 50 μ M PD98059 MEK inhibitors. Cells were lysed and either quantified using R&D Systems' phospho-ERK2 (T185/Y187) DuoSet IC ELISA (Catalog # DYC1483) or immunoblotted using R&D Systems' rabbit anti-phospho-ERK1/ERK2 (Catalog # AF1018) or rabbit anti-ERK2 (Catalog # AF1230) affinity purified polyclonal antibodies.



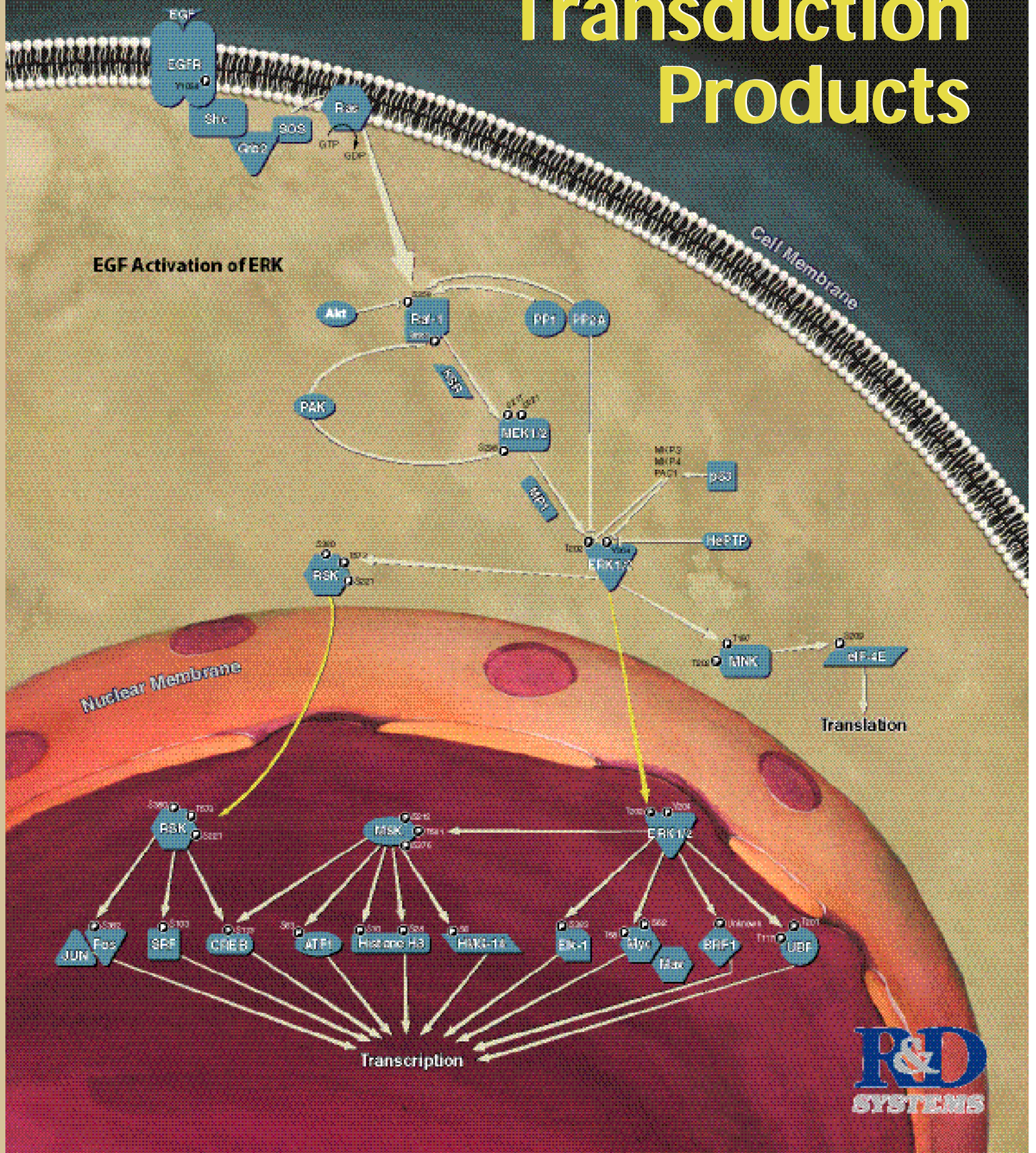
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Signal Transduction Products

EGF Activation of ERK



PHOSPHO-SPECIFIC ANTIBODIES

ANTIBODY	SPECIES	APPLICATIONS	CATALOG #
Akt (T308)	human/mouse	WB, IHC	AF8871
Akt (S473)	human/mouse	WB, IHC	AF887
ATM (S1981)	human/mouse/rat	WB	AF1655
BRCA1 (S1423)	human	WB	AF1386
Cdc2 (Y15)	human/mouse/rat	WB	AF888
Chk2 (T68)	human	WB	AF1626
ERK1/ERK2 (T202/Y204)	human/mouse/rat	WB, IHC	AF1018
GSK-3β (S9)	human/mouse/rat	WB	AF1590
JNK (T183/Y185)	human/mouse/rat	WB, IHC	AF1205
MSK1 (S212)	human	WB	AF1036
MSK1 (S376)/MSK2 (S360)	human	WB	AF1094
MSK2 (S196)	human/mouse	WB	AF189
mTOR (S2448)	human	WB	AF1665
p27 (T157)	human	WB	AF1555
p38 MAPK (T180/Y182)	human/mouse/rat	WB, IP, IHC	AF869
p53 (S15)	human/mouse/rat	WB, IHC	AF1043
p53 (S46)	human	WB	AF1489
p70 S6 Kinase (T229)	human	WB	AF896
	human	WB	AF8961
p70 S6 Kinase (T389)	human	WB	AF8963
p70 S6 Kinase (T421/S424)	human/mouse/rat	WB	AF8965
Rad17 (S635)	human	WB	AF1374
RSK (S380)	human/mouse/rat	WB, IHC	AF889
RSK (T573)	human/mouse	WB, IHC	AF8891
RSK1 (S221)/RSK2 (S227)	human/mouse/rat	WB, IHC	AF892
RSK3 (S218)	human/mouse	WB, IHC	AF893

ANTIBODY	APPLICATIONS	CATALOG #
phospho-Tyrosine	WB, IP, IHC	MAB1676
	WB, IHC	MAB16761
phospho-Tyrosine, Agarose	IP	AGM1676
phospho-Tyrosine, AP	WB, ELISA	APM1676
phospho-Tyrosine, Biotin	WB, ELISA	BAM1676
phospho-Tyrosine, HRP	WB, ELISA	HAM1676

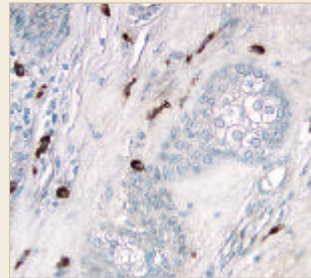


Figure 1. Detection of S473-phosphorylated Akt in paraffin-embedded human breast carcinoma tissue sections using R&D Systems' rabbit anti-phospho-Akt (S473) affinity purified polyclonal antibody (Catalog # AF887). Tissue was stained using R&D Systems' Rabbit HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS005; brown) and counterstained with hematoxylin (blue).

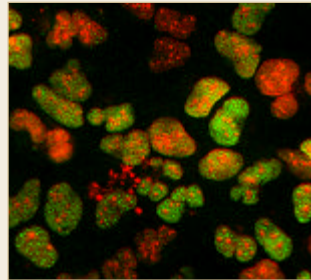


Figure 2. Detection of S15-phosphorylated p53 in human osteosarcoma cells (U2-OS cell line treated for 4 hours with 1 μ M Camptothecin) using R&D Systems' rabbit anti-phospho-p53 (S15) affinity purified polyclonal antibody (Catalog # AF1043; green). Cells were counterstained with propidium iodide (red).

Key: WB Western blot IP Immunoprecipitation IHC Immunohistochemistry

RELATED ANTIBODIES

ANTIBODY	SPECIES	APPLICATIONS	CATALOG #
Akt1	human/mouse/rat	WB	MAB1775
Akt3	human	WB	MAB1463
β-Catenin	human/mouse/rat	WB, IHC	AF1329
	human/mouse/rat	WB	MAB1329
Chk1	human/mouse/rat	WB, IP	AF1630
Chk2	human	WB	AF1358
ERK1/ERK2	human/mouse/rat	WB, IHC	MAB1576
	human/mouse/rat	WB	AF1576
ERK1/MAPK3	human	WB	AF1879
	human	WB	BAF1879
	human/mouse/rat	WB, IHC	AF1575
ERK2/MAPK1	human/mouse/rat	WB, IHC	AF1230
	human/mouse/rat	WB, IHC	MAB1230
JNK (pan-specific)	human/mouse/rat	WB, IHC	AF1387
JNK1/MAPK8	human/mouse/rat	WB	MAB1776
JNK2/MAPK9	human/mouse/rat	WB	AF1846
MKK6	human/mouse/rat	WB	AF1604
	human/mouse/rat	WB	AF16041
mTOR	human/mouse/rat	WB	AB1537

ANTIBODY	SPECIES	APPLICATIONS	CATALOG #
Nbs1	human/mouse/rat	WB	MAB1573
	human/mouse	IP	MAB15731
p21/CIP1/CDKN1A	human	WB, IP	AF1047
	human	WB, IP, IHC	MAB1047
p38α/MAPK14	human/mouse/rat	WB, IHC	AF8691
	human/mouse/rat	WB, IHC	MAB869
p38γ/MAPK12	human/mouse/rat	WB, IHC	AF1347
	human/mouse/rat	WB	AF1644
	human/mouse/rat	WB	MAB1347
p38δ/MAPK13	human	WB, IHC	AF1519
	human	WB	MAB1519
p53	human/mouse/rat	WB, IP	AF1355
	human/mouse/rat	WB, IP	MAB1355
p53, HRP	human/mouse/rat	WB	HAF1355
p53, Agarose	human/mouse/rat	IP	GAF1355
p70 S6 Kinase	human/mouse/rat	WB, IHC	AF8962
	human/mouse/rat	WB, IHC	AF8964
	human/mouse/rat	WB	MAB8962
RSK1	human/mouse/rat	WB, IHC	AF992
RSK2	human/mouse/rat	WB, IHC	AF1518

Key: WB Western blot IP Immunoprecipitation IHC Immunohistochemistry

Active and Total PTP1B DuoSet® IC ELISA Development Systems

Protein Tyrosine Phosphatase 1B (PTP1B) is a cytosolic enzyme that is an important regulator of protein phosphorylation, and mediates many cellular functions, varying from growth factor responsiveness to cellular adhesion. Alterations in its activity have been implicated in diseases such as diabetes and cancer. Antisense oligonucleotides against PTP1B normalize blood glucose levels in diabetic mice, suggesting that PTP1B-selective drugs could have considerable therapeutic value. The regulation of PTP1B activity has also been studied extensively because of the sensitivity of its catalytic active site cysteine to growth factor-induced oxidation.

In addition to PTP1B recombinant enzyme (Catalog # 1366-PT) and antibodies against PTP1B (Catalog # AF1366 and AF13661), R&D Systems offers DuoSet IC ELISA and Activity kits for measuring the levels of PTP1B protein (Catalog # DYC1366) and PTP1B activity (Catalog # DYC1625) in cell lysates. In the activity assay, a highly specific antibody captures PTP1B on microplate wells, and phosphatase activity is determined by the amount of phosphate liberated from a phosphopeptide substrate. The captured PTP1B assayed with this kit is sensitive to pharmacological agents, such as the protein tyrosine phosphatase inhibitor sodium orthovanadate (Figure 1). Performing the two PTP1B assays in parallel allows for the determination of PTP1B specific activity (Figure 2).

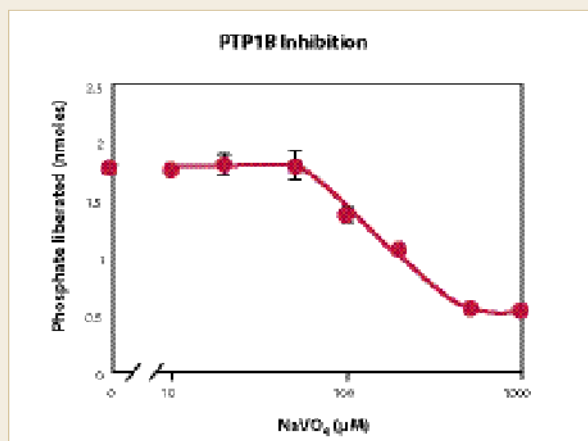


Figure 1. Measuring the inhibition of endogenous PTP1B activity by sodium orthovanadate using R&D Systems' Active PTP1B DuoSet IC assay (Catalog # DYC1625). PTP1B from MCF-7 cells was captured in microplate wells and preincubated with 10 to 1000 µM sodium orthovanadate (NaVO₄) for 5 minutes before assaying PTP1B activity. The IC₅₀ was 150 µM.

DUOSET IC ELISAs AND ACTIVITY ASSAYS	SPECIES	CATALOG #
PTEN	human/mouse/rat	DYC847
PTP1B	human	DYC1366
Active PTP1B	human	DYC1625
Malachite Green Phosphate Detection Kit		DY996
Tyrosine Phosphatase Substrate I		ES006

ANTIBODIES	SPECIES	APPLICATION	CATALOG #
Calcineurin B	human/mouse/rat	WB	AF1348
Calcineurin B	human/mouse/rat	WB	MAB1348
CD45	human	IHC, FC	MAB1430
	mouse	IP, IHC, FC	MAB114
CD45, Biotin	mouse	IP, IHC, FC	BAM114
CD45, Fluorescein	mouse	FC	FAB114F
CD45, Phycoerythrin	mouse	FC	FAB114P
PP2A	human/mouse/rat	WB	AF1653
	human/mouse/rat	WB	MAB1653
PTEN	human/mouse/rat	WB, IHC	AF847
	human/mouse/rat	WB	MAB847
PTP1B	human	WB, IHC	AF1366
	human/mouse/rat	WB	AF13661
SHP-1	human/mouse/rat	WB	AF1878
SHP-2	human/mouse/rat	WB	AF1894
VHR	human/mouse/rat	WB	MAB1654
WIP-1	human	WB, IP	2380-MC

RECOMBINANT PROTEINS	SPECIES	SOURCE	CATALOG #
Cdc25A, active	human	<i>E. coli</i>	1648-CB
Cdc25B, active	human	<i>E. coli</i>	1649-CD
PTEN, active	human	<i>E. coli</i>	847-PN
PTP1B, active	human	<i>E. coli</i>	1366-PT
VHR, active	human	<i>E. coli</i>	1654-VH

Key: WB Western blot IP Immunoprecipitation IHC Immunohistochemistry FC Flow Cytometry

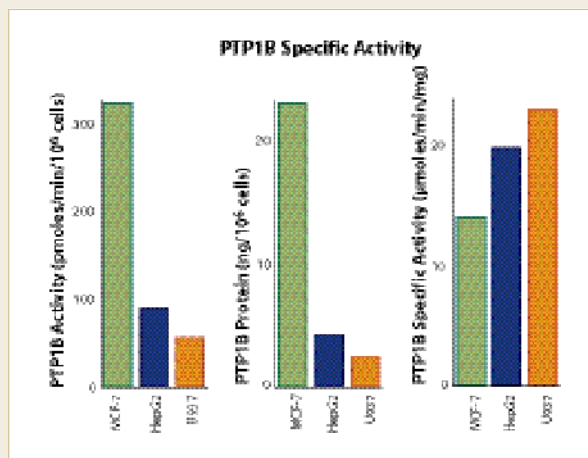


Figure 2. Comparison of PTP1B protein levels and phosphatase activity in lysates prepared from MCF-7, HepG2, and U937 cells using R&D Systems' PTP1B DuoSet IC ELISA (Catalog # DYC1366) and the Active PTP1B DuoSet IC assay (Catalog # DYC1625). Specific activity was calculated by dividing the measured tyrosine phosphatase activity by the amount of PTP1B protein present.

RECEPTOR TYROSINE KINASE ANALYSIS

EGF R Family DuoSet® IC ELISA Development Systems

The family of ErbB receptor tyrosine kinases includes four closely related members, epidermal growth factor receptor (EGF R)/ErbB1, ErbB2, ErbB3, and ErbB4. EGF R is the prototypical ErbB receptor, comprised of an extracellular ligand-binding domain, a single transmembrane domain, and a cytoplasmic protein kinase domain with intrinsic tyrosine kinase activity. The binding of members of the EGF ligand family to the extracellular domain of ErbB receptors causes the formation of receptor homo- and heterodimers, cytoplasmic kinase domain activation, and subsequent phosphorylation of specific tyrosine residues on the receptor itself as well as on intracellular signaling molecules immediately downstream. R&D Systems' Human phospho-ErbB2 DuoSet IC ELISA (Catalog # DYC1768) provides a more rapid, economical, quantitative (Figure 1), and sensitive (Figure 2) alternative to Western blots for specifically (Figure 3) measuring phosphorylated ErbB2.

DUOSET IC ELISAs	SPECIES	CATALOG #
phospho-EGF R	human	DYC1095
Total EGF R	human	DYC1854
phospho-ErbB2	human	DYC1768
phospho-ErbB3	human	DYC1769
Total ErbB3	human	DYC234
phospho-IGF 1R	human	DYC1770
phospho-PDGF Rβ	human	DYC1767
phospho-VEGF R2	human	DYC1770
Total VEGF R2	human	DYC1780

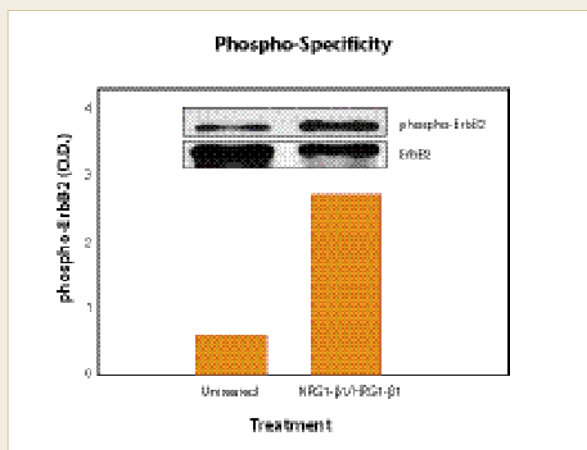


Figure 1. Lysates (100 µg) were prepared from MDA-MB-453 cells that were treated for 5 minutes with 100 ng/mL R&D Systems' recombinant human NRG1-β1/HRG1-β1 (Catalog # 396-HB). Ligand-induced phosphorylation of human ErbB2 was measured using R&D Systems' Human phospho-ErbB2 DuoSet IC ELISA (Catalog # DYC1768). Phospho-ErbB2 was detected in the same lysates by immunoprecipitation-Western blot using R&D Systems' biotinylated mouse anti-phospho-Tyrosine monoclonal antibody (Catalog # BAM1676). The blot was stripped and total ErbB2 (ErbB2) was detected using R&D Systems' biotinylated goat anti-ErbB2 affinity purified polyclonal antibody (Catalog # BAF1129).

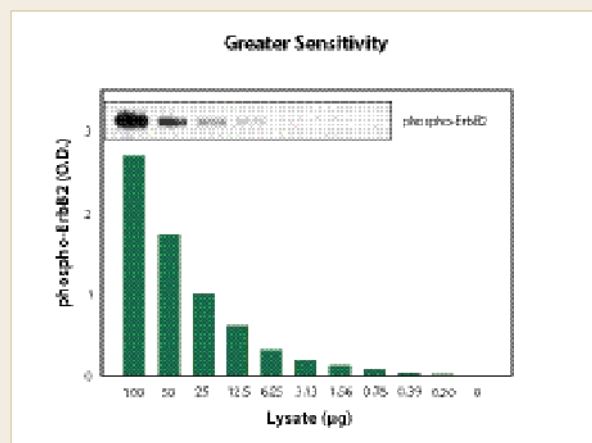


Figure 2. The sensitivity of R&D Systems' phospho-ErbB2 DuoSet IC ELISA (Catalog # DYC1768) was compared to immunoprecipitation-Western blot. Lysates were prepared as in Figure 1. R&D Systems' biotinylated mouse anti-phospho-Tyrosine monoclonal antibody (Catalog # BAM1676) was used in Western blot to detect immunoprecipitated phospho-ErbB2.

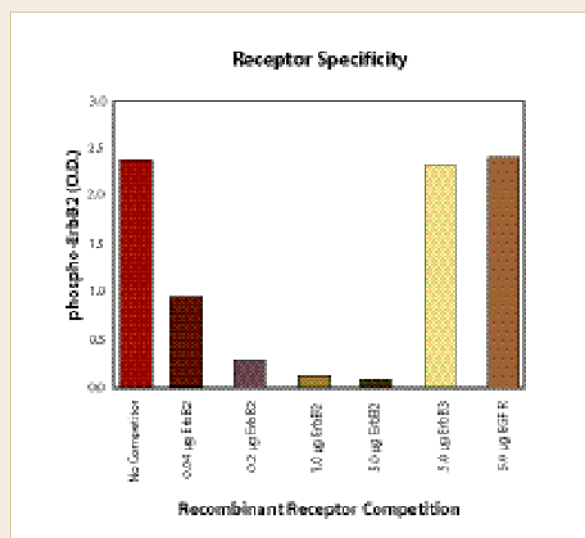


Figure 3. Specificity is demonstrated by competition of endogenous phosphorylated ErbB2 with recombinant receptors. Lysates were prepared as in Figure 1. The indicated amounts of recombinant ErbB2 (Catalog # 1129-ER), ErbB3 (Catalog # 348-ER) or EGF R (Catalog # 1095-ER) were added to 50 µg lysate and analyzed using R&D Systems' Human phospho-ErbB2 DuoSet IC ELISA (Catalog # DYC1768).

For an up-to-date listing or more information on Signal Transduction products, please visit www.RnDSystems.com/SignalTrans

