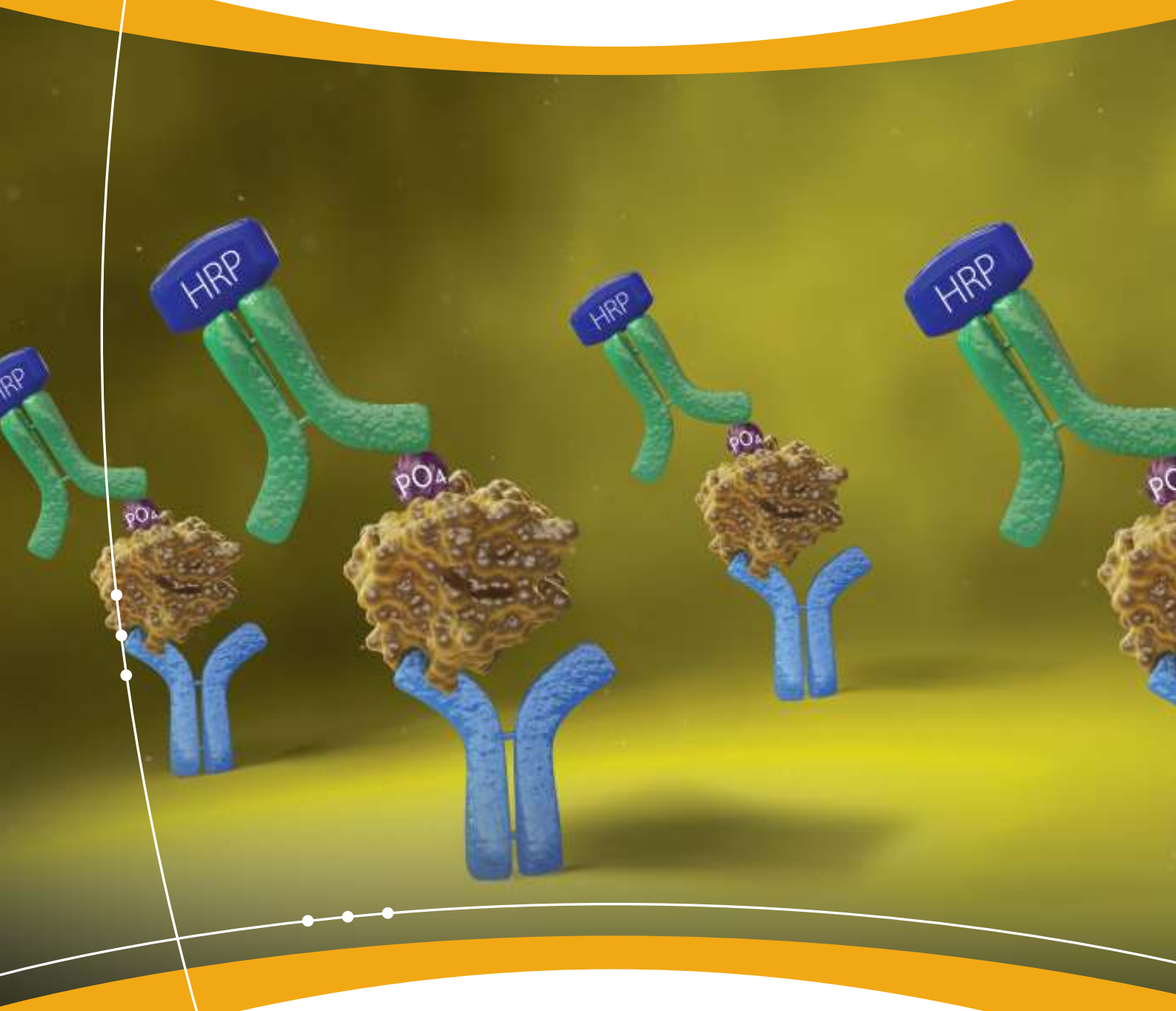
eBioscience®
high performance immunoassays

InstantOne™ ELISA

1-hour/1-wash Phospho ELISA



eBioscience
high performance immunoassays

eBioscience is committed to developing and manufacturing high-quality, innovative reagents in an ISO certified facility. As a provider of more than 10,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in the areas of innovation, quality and value.

Table of Contents

InstantOne™ ELISA Technology

Evolution in Immunoassay Sandwich ELISA

The evolution of phospho ELISAs leaps forward with the efficient sensitivity and flexibility of InstantOne ELISAs.

- **Fast:** Less than 1/3 the time of traditional ELISAs
- **Easy:** 1/4 the effort with only 1 wash step
- **Flexible:** Enables the detection of total and/or phospho protein levels of a single protein up or down a pathway on a single plate
- **Sensitive:** Meets or exceeds industry standards with only 2-3,000 cells needed per well
- **Compatible:** Colorimetric detection; utilizes standard plate readers
- **Complete:** Over 30 kits available, with more coming soon

Assay Principle

InstantOne ELISA assays use the traditional sandwich ELISA format, but with a major difference. InstantOne allows for greater flexibility, ease of use, and reduced assay time by allowing the target analyte to bind to both of the two sandwich ELISA antibodies in solution as the capture antibody binds to the plate through a proprietary mechanism. This allows for both the sample and the assay reagents to be added to the InstantOne ELISA assay plate at the same time. Unbound assay reagents and non-specific sample components are washed away just as in a traditional sandwich ELISA, while the specific analyte is detected though a colorimetric detection. The entire process takes just over 60 minutes to complete.

In addition to the ease that the 1-hour/1-wash InstantOne ELISA provides, it also adds a layer of flexibility not readily accessible with traditional sandwich ELISAs. As the antibodies are not pre-coated in the wells, several different targets can be analyzed simultaneously on the same plate in different wells. Simply add the sample lysate to the plate wells and add different antibody reagent cocktails to the different wells. Analysis of both total and phosphorylated targets across family members or down pathways in the same plate has never been easier.

1 Product Overview

InstantOne™ ELISA Kits						
	Single Target Assays			Multi-Target Assays		
Targets	Total Protein ELISA	Phospho-specific ELISA		Total/Phospho Combination ELISA	Protein Family or Pathway ELISA Kits	
	Cat. No.	Cat. No.	Phosphorylation Site	Cat. No.	Cat. No.	Included Targets
ERK 1/2	85-86011-11	85-86012-11	Thr202/Tyr204, Tyr185/Tyr187	85-86013-11	85-86014-11	phospho-ERK 1/2 & phospho-AKT
					85-86015-11	phospho-ERK 1/2, phospho-p38, & phospho-JNK
					85-86018-11	phospho-ERK 1/2, phospho-AKT, & phospho-p70 S6K
AKT	85-86041-11	85-86042-11	Ser473	85-86043-11	85-86014-11	phospho-ERK 1/2 & phospho-AKT
					85-86018-11	phospho-ERK 1/2, phospho-AKT, & phospho-p70 S6K
p38	85-86021-11	85-86022-11	Thr180/Tyr182	85-86023-11	85-86015-11	phospho-ERK 1/2, phospho-p38, & phospho-JNK
JNK	85-86031-11	85-86032-11	Thr183/Tyr185	85-86033-11	85-86015-11	phospho-ERK 1/2, phospho-p38, & phospho-JNK
p70 S6K	85-86051-11	85-86052-11	Thr389	85-86053-11	85-86018-11	phospho-ERK 1/2, phospho-AKT, & phospho-p70 S6K
IκBα	85-86061-11	85-86062-11	Ser32/36	85-86063-11		
NFκB p65	85-86081-11	85-86082-11	Ser536	85-86083-11		
STAT3	85-86101-11	85-86102-11	Tyr705	85-86103-11		
p53	85-86121-11	85-86122-11	Ser15	85-86123-11		
GAPDH	85-86131-11					

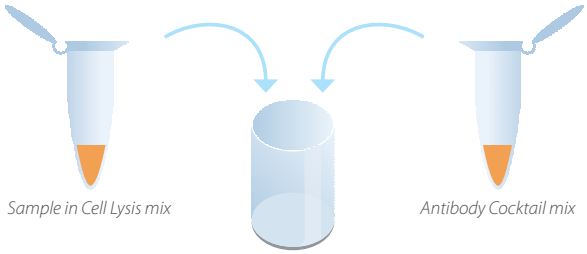
New products are launched regularly. Discover more at www.eBioscience.com.

More assays coming in 2011

Technology Comparison

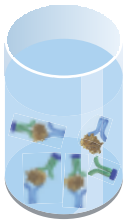
InstantOne™ ELISA

1



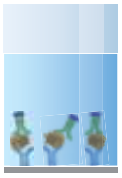
- Add lysates to the InstantOne assay microplate.
- Add Antibody Cocktail containing the capture, detection, and amplification antibodies to the microwell.
- Incubate wells for 1 hour.

2



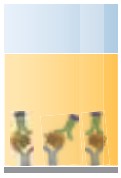
- Wash microplate with included Wash Buffer.

3



- Add colorimetric Detection Reagent to each of the used assay wells in the microplate.
- Let develop (~10-30 min).

4



- Stop the colorimetric reaction with addition of Stop Solution.
- Read in plate reader at 450/650 nm.

Key


Detection Antibody


Analyte


Capture Antibody

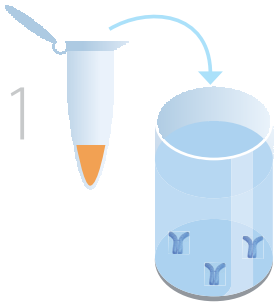

HRP


Streptavidin


Biotin

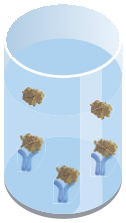
Traditional Sandwich ELISA

1



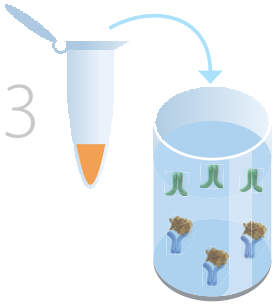
- Add Sample.
- Incubate for 1 hour.

2



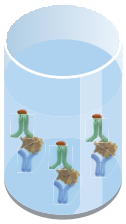
- Wash un-bound, non-specific material from well.

3



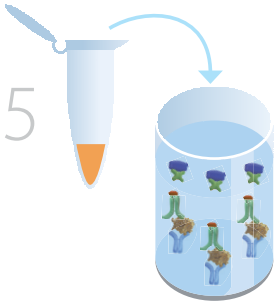
- Add detection antibody.
- Incubate for 1 hour.

4



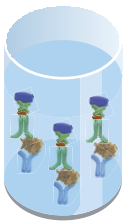
- Wash un-bound detection antibody from well.

5



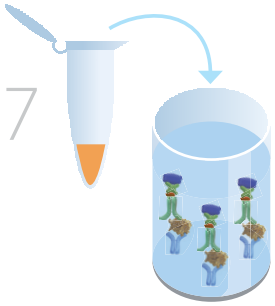
- Add HRP-conjugated Streptavidin.
- Incubate 30 min.

6



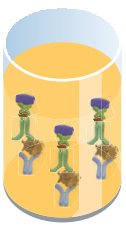
- Wash un-bound HRP-conjugated Streptavidin from wells.

7



- Add colorimetric Detection Reagent to each used assay well in the microplate.
- Let develop (~10-30 min).

8



- Stop the colorimetric reaction with addition of Stop Solution.
- Read in plate reader at 450/650 nm.

3

ERK 1/2

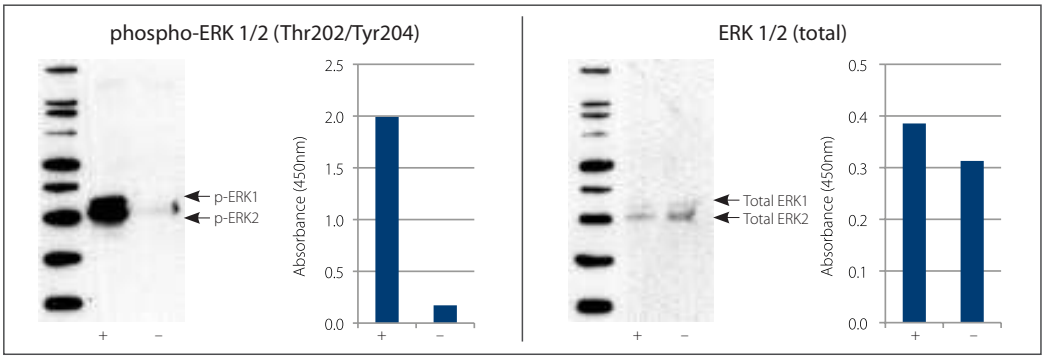
p42/p44, MAP Kinase, MAPK, ERK1, ERK2

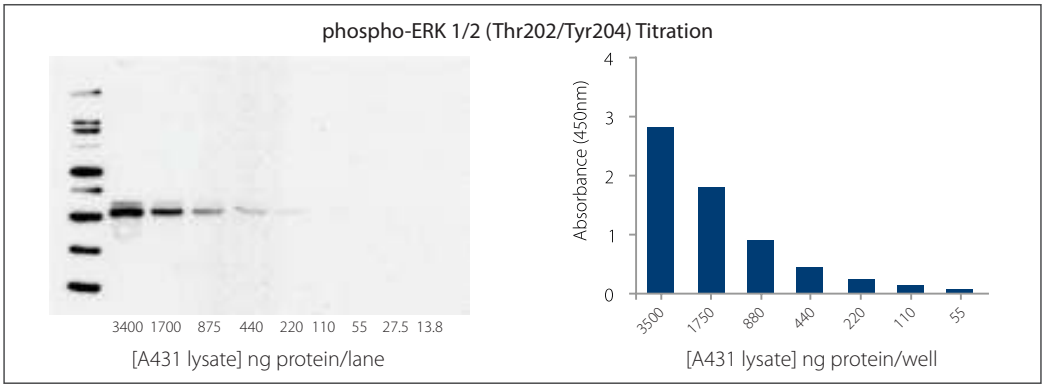
Extracellular signal-regulated kinase (ERK) is the founding member and a key component of the classical Mitogen-Activated Protein Kinase (MAPK) pathway. ERK1 (p44, MAPK3) and ERK2 (p42, MAPK2) are both activated via the MAPK/ERK pathway, which is downstream of various Receptor Tyrosine Kinases (RTKs) or GPCRs. In the classical MAPK pathway, ligand binding induces the activation of an RTK that initiates a signaling cascade that results in activation of the GTPase Ras and the Ser/Thr kinase Raf (MAPKKK). Raf then binds to and activates MEK (MAPKK) via phosphorylation. MEK, a Ser/Thr and Tyr kinase, activates ERK by phosphorylation of its TxY motifs, namely Thr202/Tyr204 and Thr185/Tyr187 of ERK1 and ERK2, respectively. Both phosphorylation sites are required for ERK activation and no known mutations exist that cause constitutive ERK1/2 activation. As such, detection of ERK1/2 phosphorylation is frequently used to assess its activation, as well as that of MEK, a popular drug target. Once activated, ERK phosphorylates a variety of proteins that regulate cellular processes such as cell division, proliferation, survival, differentiation, apoptosis, motility, and metabolism. Due to its established critical role in these processes, the MAPK signaling pathway is a frequent target for oncology-related drug development.

ERK 1/2

Cells: *A431 cells were treated with 10 µM AG1478 (an EGFR kinase inhibitor) for 3 hours (-) or treated with 10 ng/mL EGF for 10 min (+).*

Analysis: *10 µg/analysis WB, 5 µg/analysis InstantOne.*





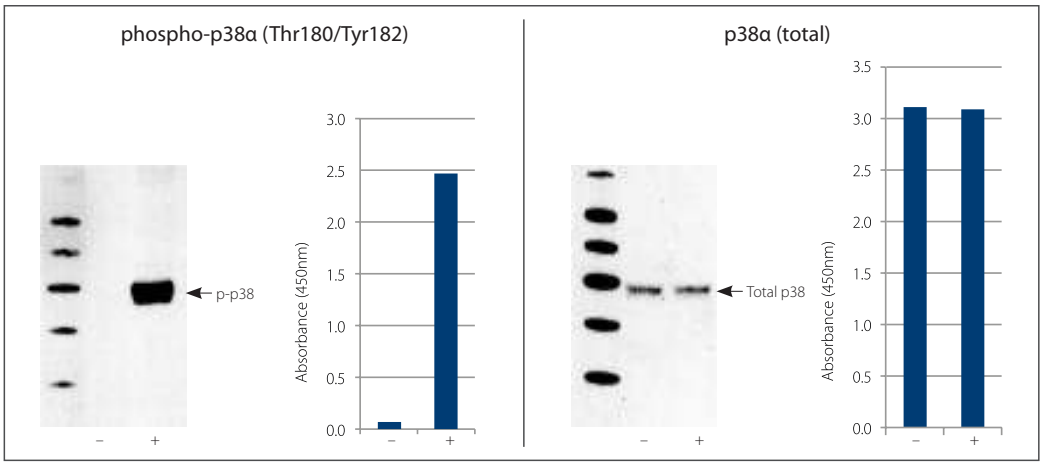
New products are launched regularly. Discover more at www.eBioscience.com.

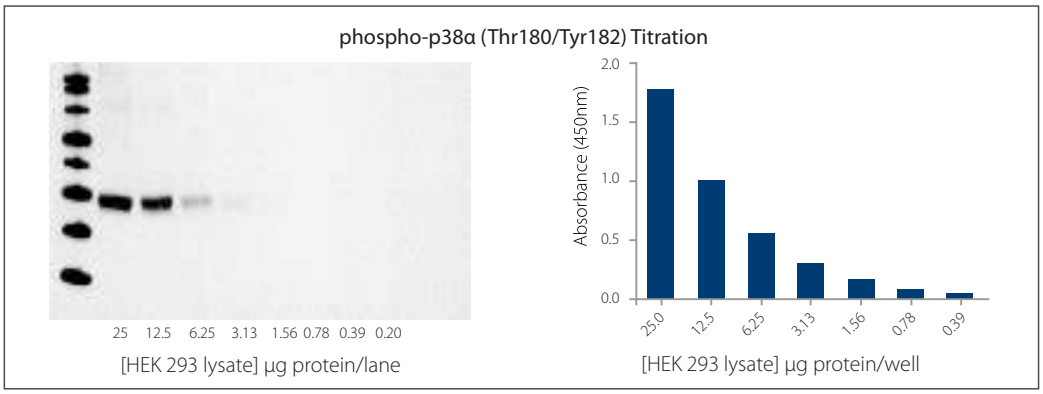
Description	Catalog Number
ERK 1/2 (Total) InstantOne™ ELISA	85-86011
phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187) InstantOne™ ELISA	85-86012
ERK 1/2 (Total/Phospho) InstantOne™ ELISA	85-86013
MAPK Family (ERK, p38, JNK) Activation InstantOne™ ELISA	85-86015
AKT/ERK Activation InstantOne™ ELISA	85-86014
Erk/AKT/p70 S6K Activation InstantOne™ ELISA	85-86018

p38α

SAPK2A, Stress-activated protein kinase 2α, MAPK14, Mitogen-activated protein kinase 14

p38 MAPKs, also known as the Stress-Activated Protein Kinases (SAPKs), are a sub-family of the JNK/SAP family of MAP Kinases. There are four isoforms of p38 MAPK, denoted α (SAPK2A), β (SAPK2B), γ (SAPK3b) and δ (SAPK4). Like ERK and JNK, p38 MAP Kinases are activated via phosphorylation of the TxY motif that corresponds to Thr180/Tyr182 for p38α. p38 is activated by MKK3 and MKK6 in response to cellular stresses, most notably inflammatory cytokines, irradiation, UV light, osmotic shock, lipopolysaccharides, and toxins such as anisomycin. Once activated, p38 phosphorylates numerous targets, including the transcription factors ATF2 and ELK1 and kinases such as MAPKAPK2. Thus, p38 plays a critical role in the production of cytokines including IL-6, inflammation, cancer, and neurodegenerative diseases.





p38 MAPK

Cells: *HEK293 cells untreated (-) or treated with 2 µg/mL anisomycin for 30 min (+).*

Lysis: *Lysis Mix for 10 min with shaking.*

Analysis: *50 µg/analysis*

Description	Catalog Number
p38 MAPK (Total) InstantOne™ ELISA	85-86021
phospho-p38 MAPK (Thr180/Tyr182) InstantOne™ ELISA	85-86022
p38 MAPK (Total/Phospho) InstantOne™ ELISA	85-86023
MAPK Family (ERK, p38, JNK) Activation InstantOne™ ELISA	85-86015

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JNK 1/2/3

c-Jun N-Terminal Kinase 1/2, MAPK8/9, Stress-activated protein kinase JNK1/2, SAPK 1/2

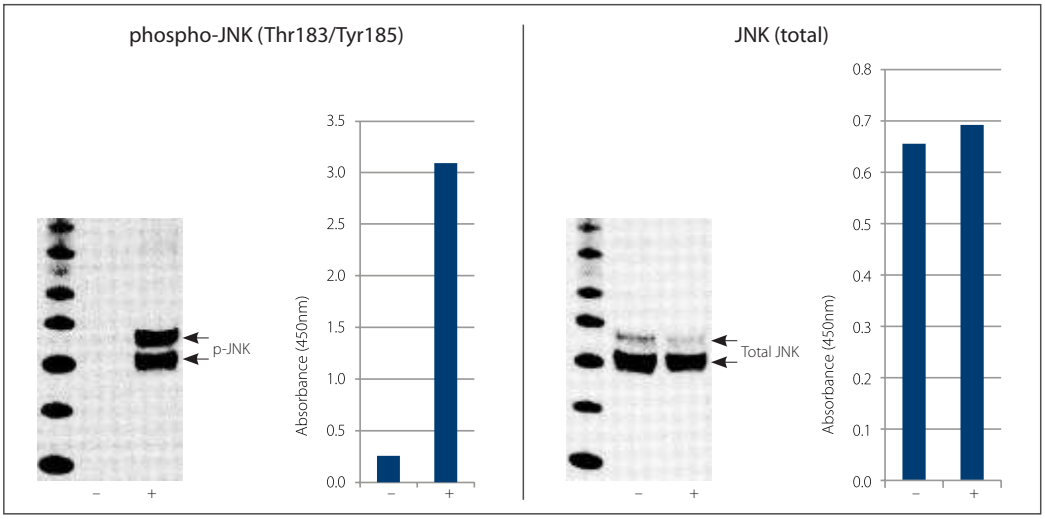
Jun N-terminal Kinases (JNKs) are members of the Mitogen-Activated Protein Kinase (MAPK) and Stress-Activated Protein Kinase 1 (SAPK1) family. JNK is phosphorylated on the TxY motif at residues Thr183/Tyr185 in response to environmental stress and pro-inflammatory cytokines. This activation results in the phosphorylation of many transcription factors including components of the AP-1 family (e.g., Jun) and ATF2. JNK phosphorylation is also required for Th1 cell differentiation.

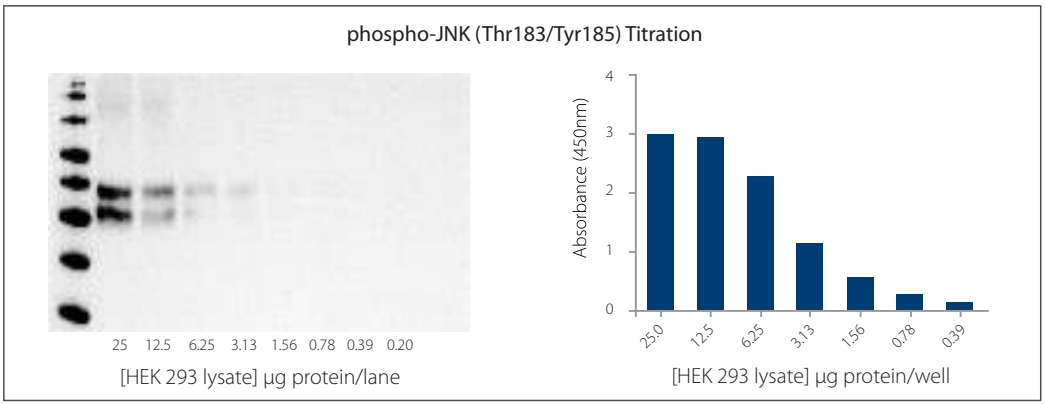
JNK 1/2/3

Cells: *HEK293 cells untreated (-) or treated with 2 µg/mL anisomycin for 30 min (+).*

Lysis: *1 mL Lysis Mix for 10 min with shaking.*

Analysis: *25 µg/analysis.*





Description	Catalog Number
JNK 1/2/3 (Total) InstantOne™ ELISA	85-86031
phospho-JNK 1/2/3 (Thr183/Tyr185) InstantOne™ ELISA	85-86032
JNK (Total/Phospho) InstantOne™ ELISA	85-86033
MAPK Family (ERK, p38, JNK) Activation InstantOne™ ELISA	85-86015

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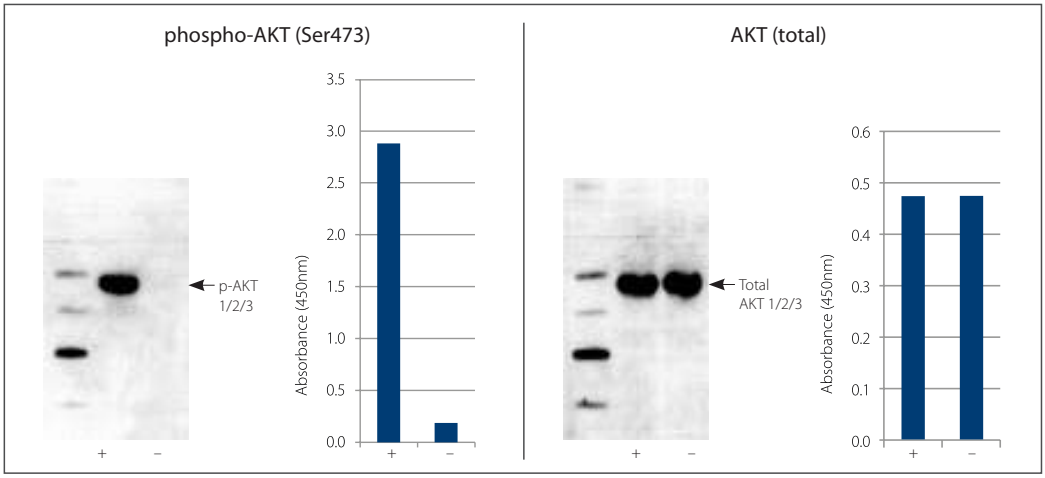
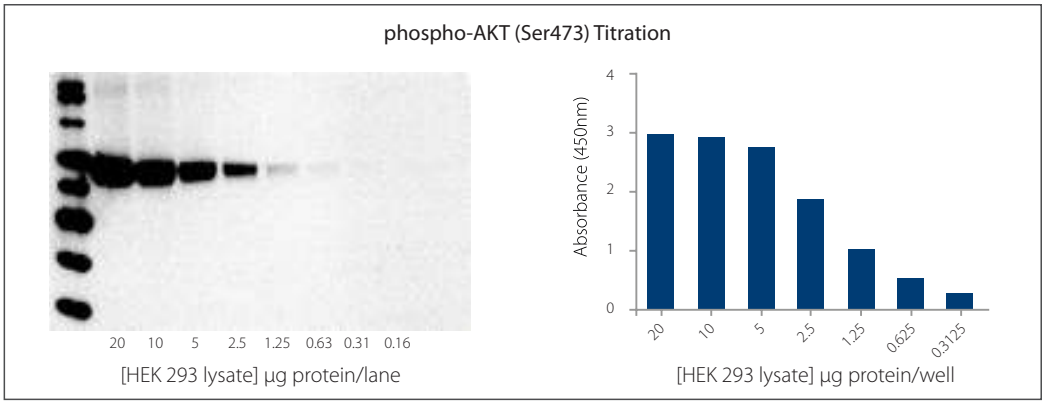
AKT

PKB, Protein Kinase B, RAC-PK-alpha

AKT is one of the principle kinases activated by phosphoinositide 3-kinase (PI3K). Activation of PI3K results in the generation of phosphatidylinositol (3,4,5)-triphosphate (PIP3). These lipid second messengers bind to the pleckstrin homology domain of AKT to promote its translocation to the plasma membrane for activation via phosphorylation at Thr308 and Ser473 by PDK1 and the mTOR TORC2 complex, respectively. Phosphorylation at both these sites is required for full activation of AKT Ser/Thr kinase activity. AKT phosphorylates over 50 known substrates, including GSK3, AS160, PRAS40, TSC 1, TCS 2, Raf-1, Bad, the FOXO family of transcription factors, and PFK2. Due to its established critical role in numerous biological processes, AKT is a frequent target for oncology-related drug development.





AKT 1/2/3

Cells: *MCF7 cells treated with 10µM wortmannin for 2 hours (-) or treated with 10 ng/mL insulin for 10 min (+).*

Lysis: *10 min at RT with shaking.*

Analysis: *10 µg/analysis.*

Description	Catalog Number
AKT 1/2/3 (Total) InstantOne™ ELISA	85-86041
phospho-AKT 1/2/3 (Ser473) InstantOne™ ELISA	85-86042
AKT (Total/Phospho) InstantOne™ ELISA	85-86043
AKT/ERK Activation InstantOne™ ELISA	85-86014
Erk/AKT/p70 S6K Activation InstantOne™ ELISA	85-86018

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p70 S6K

Ribosomal protein S6 kinase beta-1, RPS6KB1, STK14A

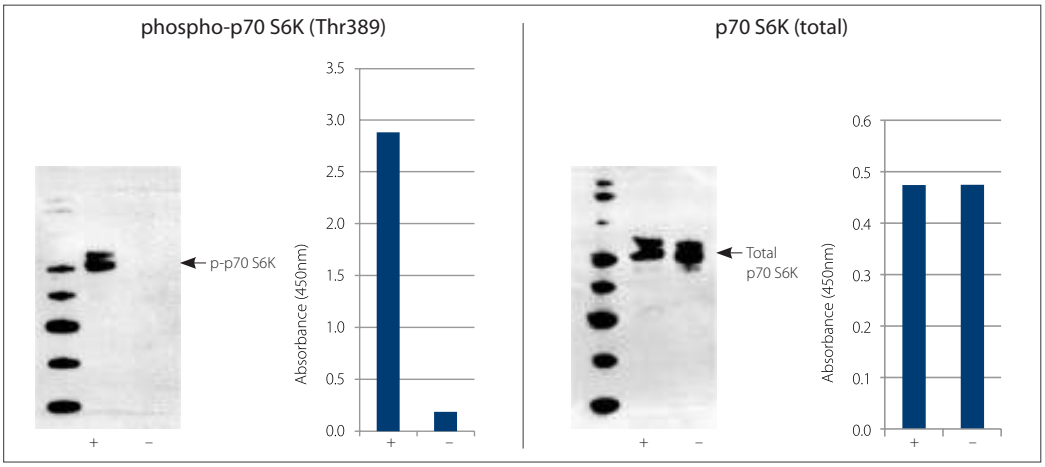
p70 S6 Kinase (p70S6K) is a member of the ribosomal S6 kinase family of Ser/Thr kinases. p70 S6K activity is controlled by multiple signaling pathways, including the MAPK, phosphoinositide-3 kinase (PI3K), and mTOR pathways. This regulation results in the phosphorylation of multiple sites of p70 S6K, including Thr389 within the linker domain, which is required for full activation. Activated p70 S6K phosphorylates several residues on the S6 ribosomal protein, thereby leading to increased protein synthesis.

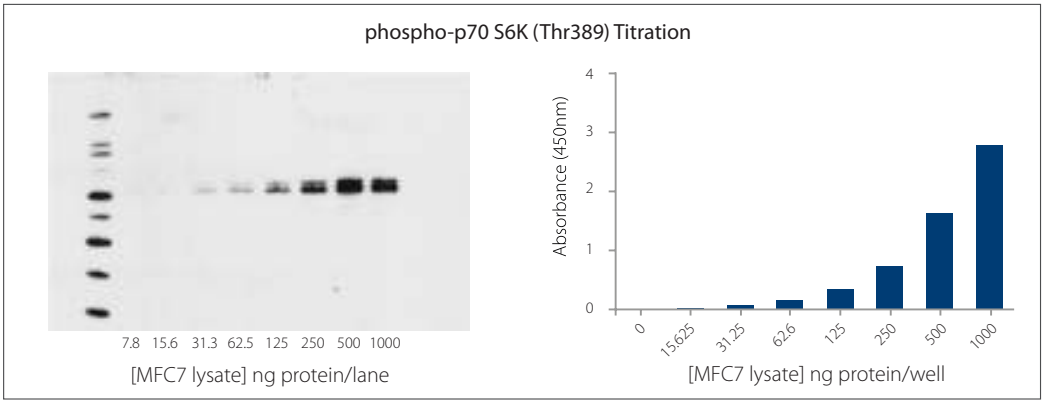
p70S6K

Cells: *MCF7 cells treated with 10 µM wortmannin for 3 hours (-) or treated with 10 ng/mL insulin for 10 min (+).*

Lysis: *10 min at RT with shaking.*

Analysis: *1 µg/analysis.*





Description	Catalog Number
p70 S6K (Total) InstantOne™ ELISA	85-86051
phospho-p70 S6K (Thr389) InstantOne™ ELISA	85-86052
p70 S6K (Total/Phospho) InstantOne™ ELISA	85-86053

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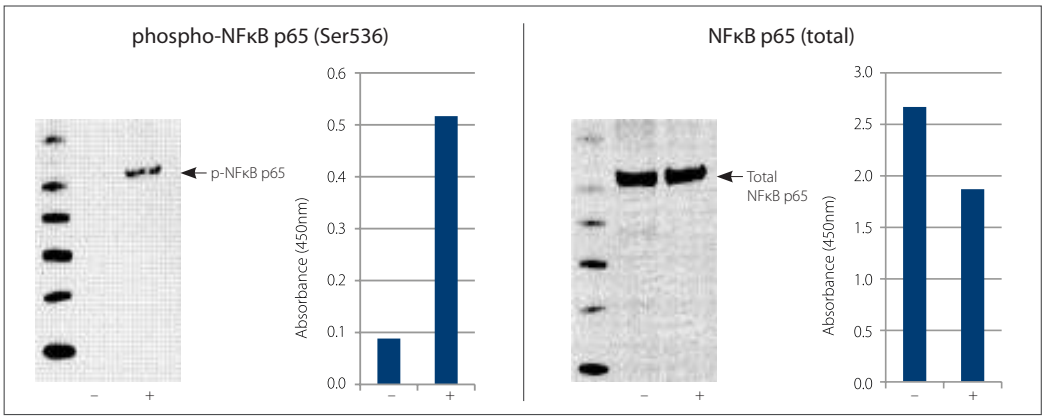
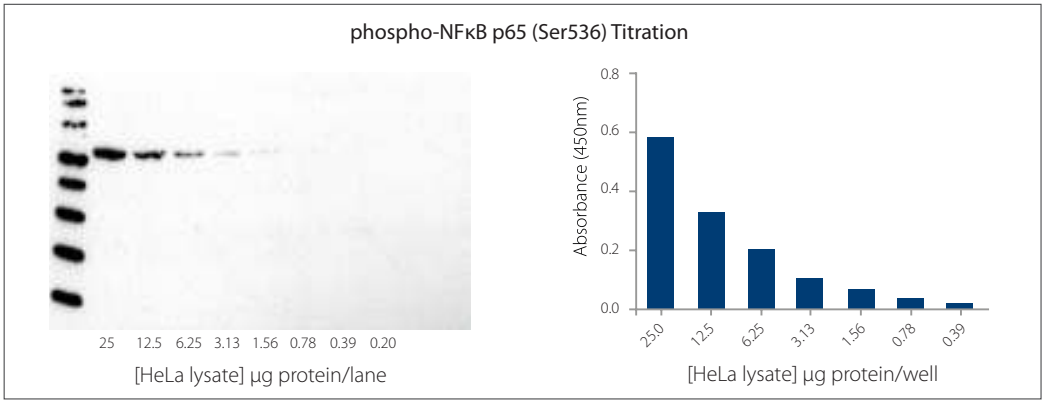
NFκB p65

Nuclear Factor Kappa B, p65 subunit, RelA, NFKB3

Nuclear Factor kappa B (NFκB) constitutes a family of transcription factors consisting of five members, including Rel (c-Rel), RelA (p65), RelB, NFκB1 (p50 and its precursor p105), and NFκB2 (p52 and its precursor p100). NFκB members can exist as either homo- or heterodimers. NFκB dimers containing p65 are activators of transcription. In unstimulated cells, NFκB is inactive and retained in the cytoplasm due to binding by inhibitory IκB proteins. Upon stimulation by inducers such as TNFα, IL-1, or PMA, IκBα is phosphorylated and degraded. This results in the release of the NFκB complex from the IKK complex and cleavage of the p105 subunit into its active p50 form. Subsequently, the p50/p65 complex translocates to the nucleus where it activates transcription. NFκB regulates the expression of numerous genes, including IκBα, cytokines, chemokines, adhesion targets, and acute phase proteins involved in growth, development, apoptosis, immune and inflammatory response, and activation of various viral promoters.





NF-κB p65

Cells: *HeLa cells untreated (-) or treated with 10 ng/mL TNFα for 10 min (+).*

Lysis: *1 mL Lysis Mix for 10 min with shaking.*

Analysis: *20 µg/analysis.*

Description	Catalog Number
NFκB p65 (Total) InstantOne™ ELISA	85-86081
phospho-NFκB p65 (Ser536) InstantOne™ ELISA	85-86082
NFκB p65 (Total/Phospho) InstantOne™ ELISA	85-86083

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IκBα

I kappa B alpha

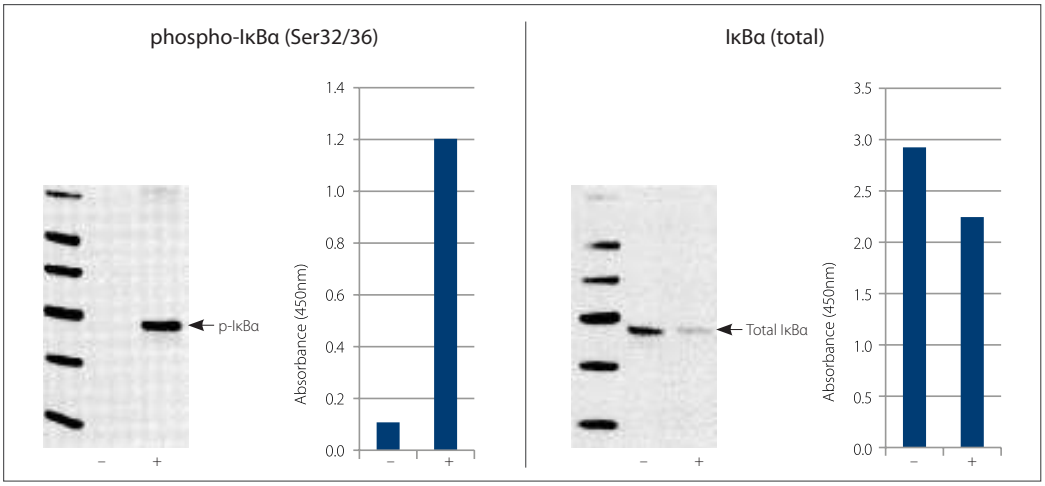
IκB proteins are present within the cytosol where they bind to the NFκB/Rel transcription factor complex to maintain its inactive state. To activate NFκB, IκB must dissociate from the complex. This is induced by phosphorylation of IκB at Ser32 and Ser36 in response to various extracellular signals, including inflammatory cytokines, growth factors, and chemokines. Phosphorylation targets IκB for ubiquitination and proteosomal degradation, thereby enabling NFκB to translocate into the nucleus to modulate gene expression. Examination of IκB phosphorylation has been used widely as an indicator of NFκB activation.

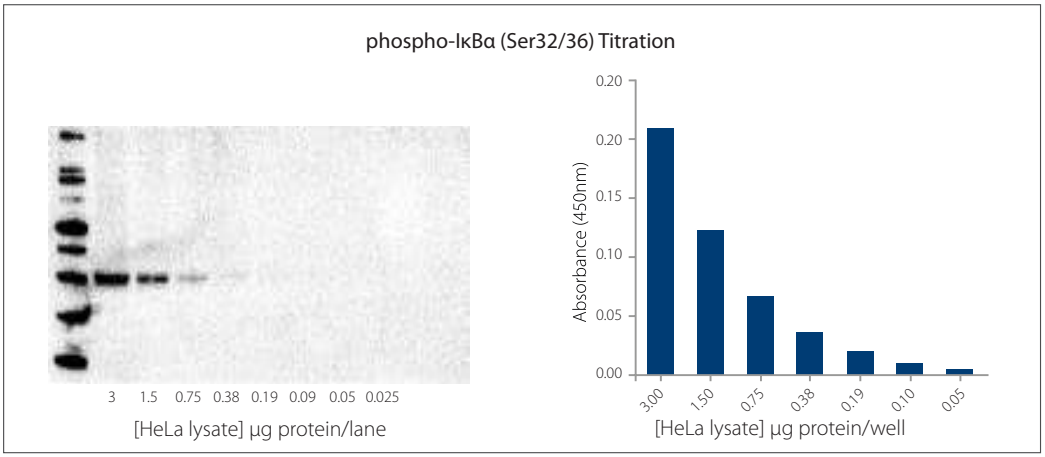
IκBα

Cells: *HeLa cells untreated (-) or treated with 10 ng/mL TNFα for 5 min (+).*

Lysis: *Lysis Mix for 10 min at RT, with shaking.*

Analysis: *10 µg/analysis.*





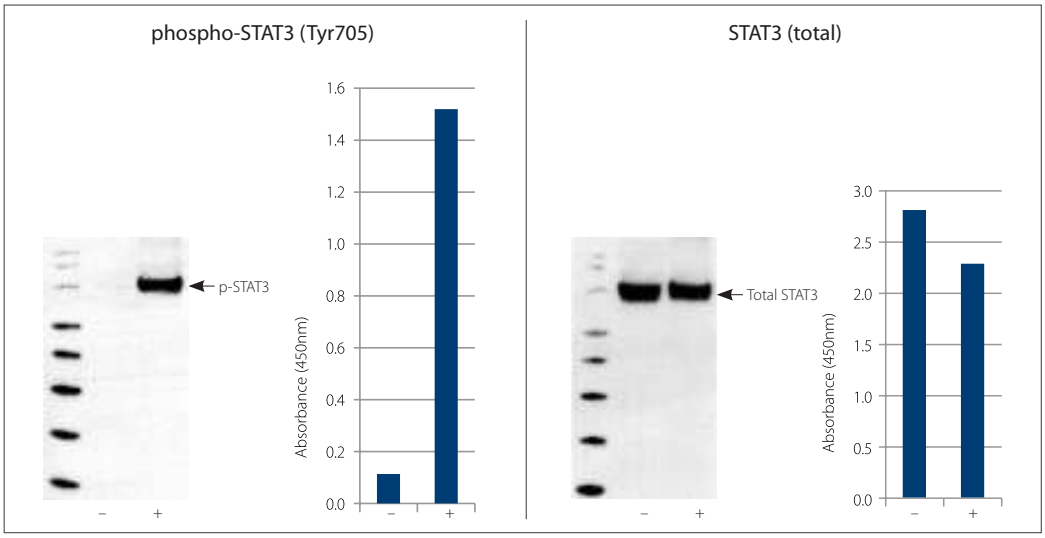
Description	Catalog Number
IκBα (Total) InstantOne™ ELISA	85-86061
phospho-IκBα (Ser32/36) InstantOne™ ELISA	85-86062
IκBα (Total/Phospho) InstantOne™ ELISA	85-86063

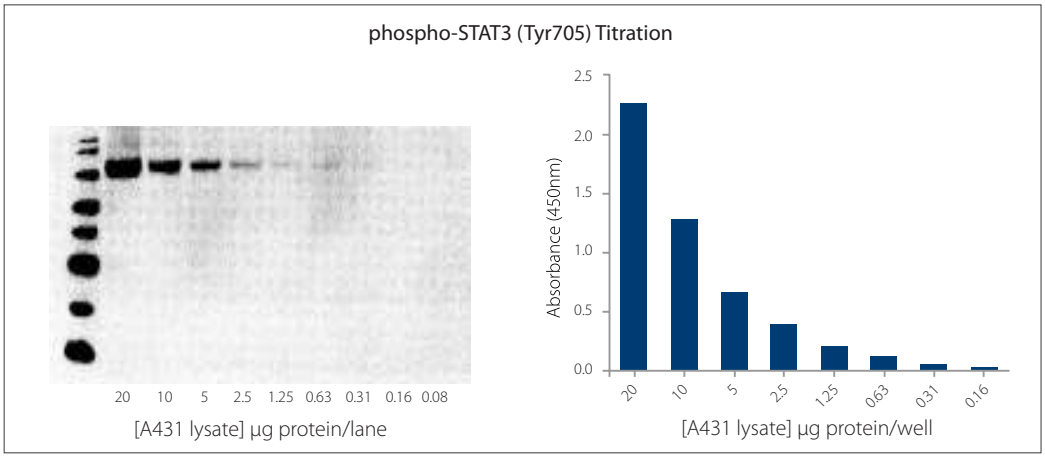
New products are launched regularly. Discover more at www.eBioscience.com.

STAT3

Signal Transducer and Activator of Transcription 3, Acute-phase response factor, APRF

STAT3 is a member of the Signal Transducers and Activators of Transcription family of transcription factors that are activated via phosphorylation by JAK tyrosine kinases of receptor tyrosine kinases in response to various stimuli, including cytokines and growth factors. Phosphorylation at Tyr705 leads to STAT3 dimerization and nuclear translocation. STAT3α, but not STAT3β, is also phosphorylated at Ser727, which enhances its transcriptional activity. STAT3 is believed to be one of the main mediators of IL-6 signaling. Moreover, STAT3 has been linked to cellular activities that are associated with tumor progression. Inhibition of STAT3 results in impaired apoptosis and cell migration, as well as upregulation of genes involved in angiogenesis and immune suppression.





STAT3

Cells: *Serum-starved A431 cells were either untreated(-) or treated with 2 µg/mL EGF for 10 min, then lysed.*

Lysis: *Lysis Mix at RT for 10 min with shaking.*

Analysis: *10 µg/analysis.*

Description	Catalog Number
STAT3 (Total) InstantOne™ ELISA	85-86101
phospho-STAT3 (Tyr705) InstantOne™ ELISA	85-86102
STAT3 (Total/Phospho) InstantOne™ ELISA	85-86103

New products are launched regularly. **Discover more at www.eBioscience.com.**

p53

TP53, Cellular tumor antigen p53

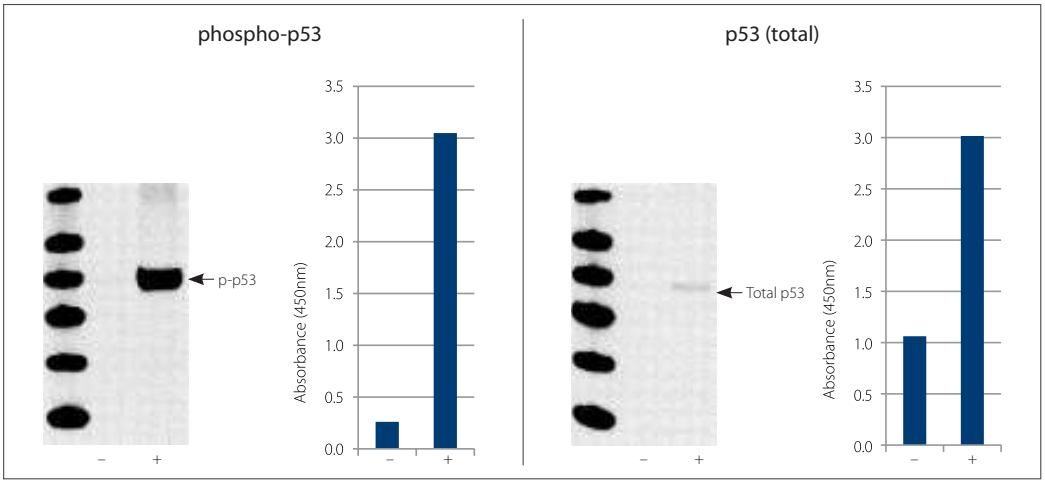
p53 is a tumor suppressor that is regulated by transcriptional, post-transcriptional, and post-translational mechanisms in response to stress. Studies demonstrate that p53 is critical for the prevention of tumorigenesis and may potentially be involved in at least half of all known cancers. p53 functions as a transcription factor to regulate genes involved in apoptosis, cell cycle, senescence, metabolism, angiogenesis, immunity, differentiation, and migration. Moreover, p53 is phosphorylated at multiple sites, including Ser15, following DNA damage.

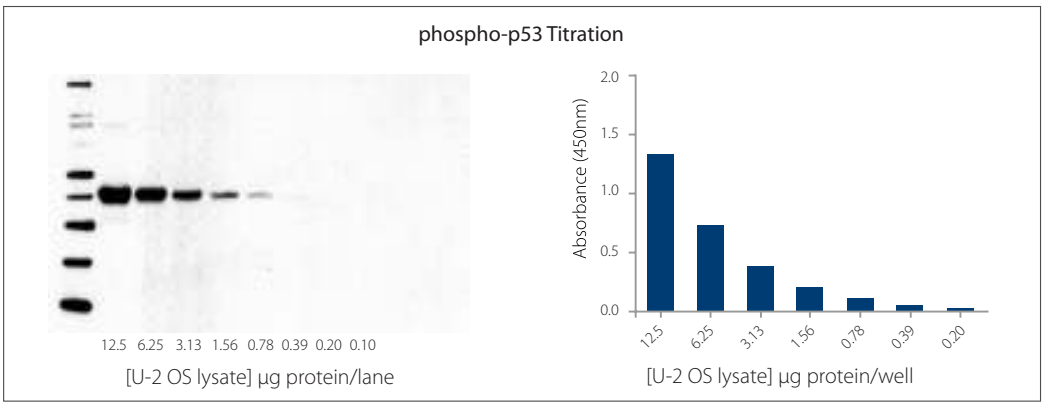
p53

Cells: *U-2 OS cells untreated (-) or treated with 10 ng/mL doxorubicin for 18 hours (+).*

Lysis: *1mL Lysis Mix for 10 min with shaking.*

Analysis: *30 µg/analysis.*





Description	Catalog Number
p53 (Total) InstantOne™ ELISA	85-86123
phospho-p53 (Ser15) InstantOne™ ELISA	85-86122
p53 (Total/Phospho) InstantOne™ ELISA	85-86121

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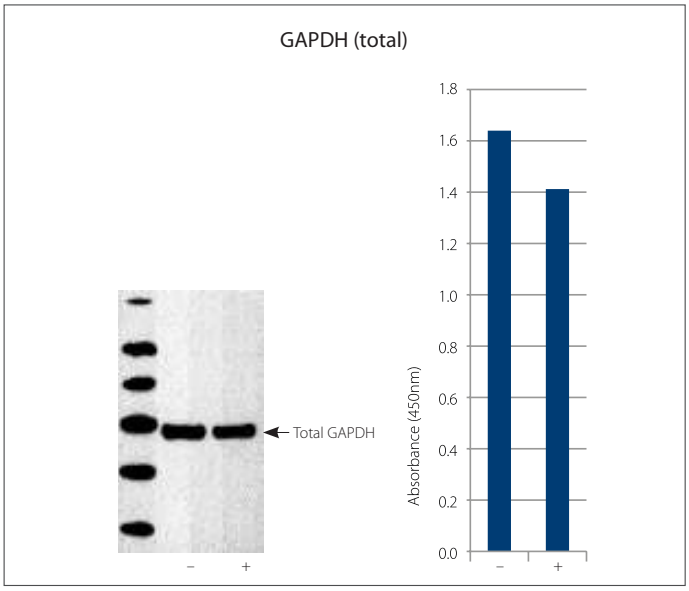
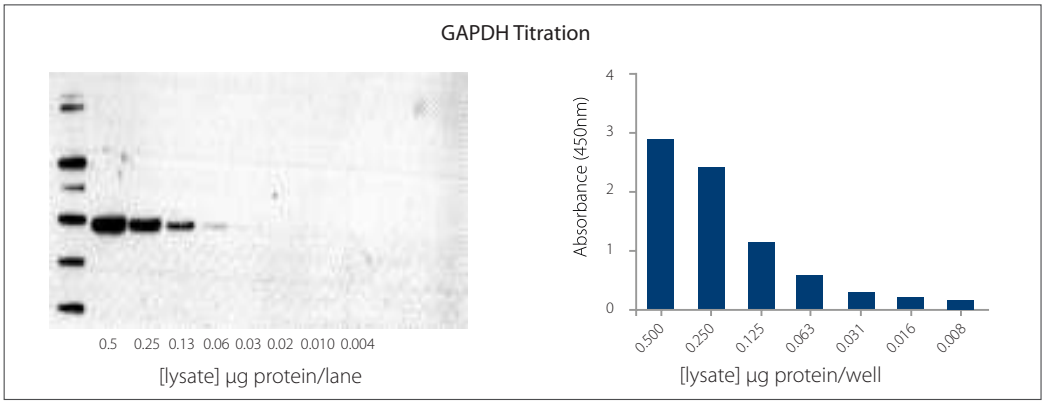
GAPDH

Glyceraldehyde-3-phosphate dehydrogenase

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a ubiquitously expressed enzyme involved in glycolysis. In addition, GAPDH plays a role in transcription, apoptosis, and vesicle transport. As an established housekeeping gene, GAPDH expression is assessed frequently as an internal loading control.





GAPDH

Cells: *HEK293 cells untreated (-) or treated with 2 µg/mL anisomycin for 30 min (+).*

Lysis: *Lysis Mix for 10 min with shaking.*

Analysis: *1 µg/analysis WB, 100 ng/analysis InstantOne.*

Description	Catalog Number
GAPDH InstantOne™ ELISA	85-86131

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