

# Anti-phospho-Erk 1/2 (Thr202/Tyr204, Thr185/Tyr187), recombinant clone AW39R

Monoclonal Antibody

pack size: 100  $\mu$ L

Cat. # 05-797R

Store at -20°C

Lot # NG1527842

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NOT FOR USE IN HUMANS



## Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IF, FC, Lum	H, M, R	IgG	Thr185/Tyr187	Rb	42 & 44 kDa	NP_002736.3

### Background

Erk (Extracellular signal-Related Kinase) is a family of two, highly homologous proteins denoted as Erk1 (p44, MAPK3) and Erk2 (p42, MAPK1) that both function in the same pathway. The two proteins are often referred to collectively as Erk1/2 or p44/p42 MAP kinase. The Erk pathway is considered the classical, canonical MAPK (Mitogen-Activated Protein Kinase) signaling pathway. It is an evolutionarily conserved pathway that controls and is a critical regulator of the growth and survival through the promotion of cell proliferation and the prevention of apoptosis. Erk is involved in the control of many fundamental cellular processes including cell proliferation, survival, differentiation, apoptosis, motility and metabolism. Erk is activated by growth factor stimulation of receptor tyrosine kinases (RTKs), GPCR, and/or integrin stimulation. This activates the Ras-Raf-MEK-Erk pathway that results in the phosphorylation/activation of Erk1/2 (p44/p42) on the TxY motif (Thr202/Tyr204 and Thr185/Tyr187 for Erk1 & Erk2, respectively).

### Presentation

Protein A purified recombinant rabbit monoclonal in storage buffer containing 60mM Tris, 108mM Glycine, pH7.5, 90mM NaCl, 0.03% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> in 40% glycerol.

### Specificity

Recognizes Erk 1 & 2 only when dually phosphorylated on its TxY activation motif.

### Species Cross-reactivity

Human, Mouse, and Rat

### Immunogen

Phosphorylated peptide encompassing the TxY motif in the activation loop of Erk1 and Erk2.

### Molecular Weight

42 and 44 kDa

### Method of Purification

Protein A purified

### Storage and Handling

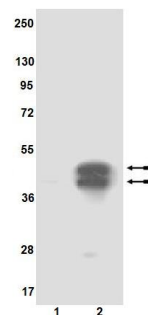
Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.

### Quality Control Testing

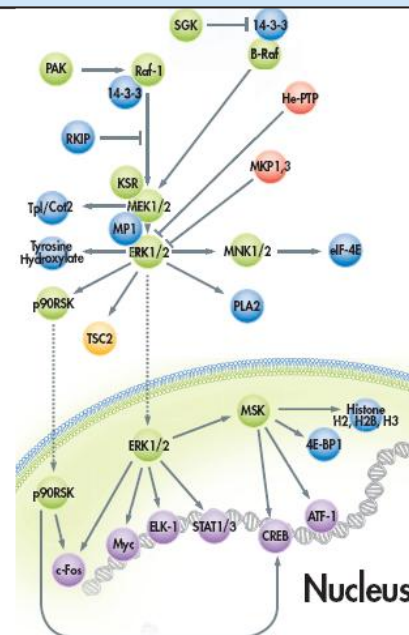
#### Western Blot Analysis:

This lot detected phosphorylated Erk1/2 at a 1:1,000 dilution in lysates from PDGF-treated NIH/3T3 cell lysate, but not their non-treated counterparts as resolved via SDS-PAGE and transferred to PVDF (Immobilon-P).



#### Western Blot Analysis:

Untreated and PDGF-treated NIH/3T3 (lanes 1 and 2, respectively) lysates were resolved by SDS-PAGE, transferred to PVDF, and probed with anti-phospho-ERK 1/2 (Thr202/Tyr204, Thr185/Tyr187), recombinant clone AW39 (1:1,000). Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrows indicates phosphorylated Erk1 (p44) and Erk2 (p42).



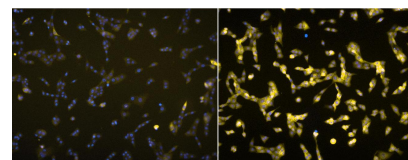
### References

- Blumer, K.J. and G.L. Johnson, Trends Biochem. Sci. **19**: 236-240, 1994.
- Boulton, T.G., et al., Science **249**: 64-67, 1990.
- Grammer, T.C. and J. Blenis, Oncogene **14**: 1635-1642, 1997.

### Additional Research Application

#### Immunofluorescence Analysis:

Untreated (left) and EGF-treated (right) A431 cells were fixed, permeabilized and dual stained with anti-phospho-Erk 1/2 (Thr202/Tyr204, Thr185/Tyr187) (Cy3, yellow) and DAPI (nuclei, blue).



**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IF Immunofluorescence FC Flow Cytometry IH Immunohistochemistry (Tissue) IH(P) Immunohistochemistry (Paraffin) LUM Luminex

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit Mk Monkey

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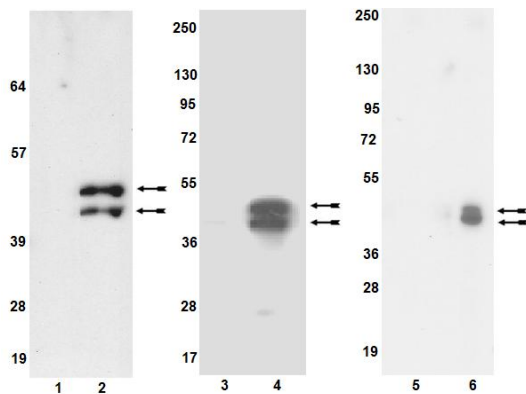
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## Anti-phospho-Erk 1/2 (Thr202/Tyr204, Thr185/Tyr187), clone AW39R

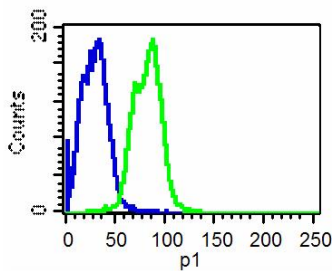
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### Western Blot Analysis:

Untreated and NGF-treated PC12 (lanes 1 & 2), untreated and PDGF-treated NIH/3T3 (lanes 3&4), and untreated and EGF-treated A431 (lanes 5&6) lysates were resolved by SDS-PAGE, transferred to PVDF, and probed with anti-phospho-Erk 1/2 (Thr202/Tyr204, Thr185/Tyr187), recombinant clone AW39R (1:1,000) (1 second exposure). Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrows indicates phosphorylated Erk1 (p44) and Erk2 (p42).



### Flow Cytometry Analysis:

Untreated (blue) and PMA-treated (20mM for 20 min) Jurkat cells were fixed, permeablized, and stained with anti-phospho-Erk 1/2 (Thr202/Tyr204, Thr185/Tyr187), recombinant clone AW39 at a 1:100 dilution.

## PROTOCOLS

### Western Blot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na<sub>3</sub>VO<sub>4</sub>; 1 mM NaF) and transfer the proteins to PVDF (Immobilon-P). Wash the blotted PVDF (Immobilon-P) twice with Tris/0.05% Tween.
2. Block the blotted PVDF in freshly prepared TBST containing 5% nonfat dry milk (Catalog # 20-200), for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with 1:1,000 of anti-phospho-Erk 1/2 (Thr202/Tyr204, Thr185/Tyr187), diluted in freshly prepared TBST-MILK at room temperature for 1 hour or overnight at 4°C with agitation.
4. Wash the PVDF 4 times with TBST for 3-5 minutes each.
5. Incubate the PVDF in the secondary reagent of choice (donkey anti-rabbit HRP conjugated IgG, Catalog # AP182P) in TBST-MILK for 1 hours at room temperature with agitation.
7. Wash the PVDF 4 times in TBST for 3-5 minutes each wash.
9. Use detection method of choice (enhanced chemiluminescence was used).

### Flow Cytometry Protocol

1. Grow and Treat cells as desired.
2. Rinse cells 1 time with cold PBS.
3. Add fixative (1% formaldehyde in PBS) for 20 minutes at room temperature.
4. Rinse cells two times with PBS.
5. Resuspend cells in 1X Permeabilization Solution (Catalog# 20-259 - solution containing 5% saponin, 100mM HEPES, pH 7.4, 1.4M NaCl, 25mM CaCl<sub>2</sub>, 0.2 µm-filtered).
6. Incubate on ice or at 4°C for 10 mins.
7. Add primary antibody (1:100 dilution for 1 million cells) in 1X Permeabilization Solution for at least 1 hour at 4°C.
8. Rinse cells once with 1X Permeabilization Solution.
9. Add secondary antibody (Catalog# AP182F at a 1:100 dilution for 1 million cells) in 1X permeabilization Solution for at least 1 hour 4°C.
10. Rinse cells twice with FACS Buffer (PBS+2% FCS+0.05% sodium azide).
11. Resuspend cells in FACS Buffer and analyze.

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**RELATED PRODUCTS (specific)**

cat #	description
17-463	■ Erk 1/2 STAR ELISA Assay kit
17-464	■ Phospho-Erk 1/2 STAR ELISA Assay kit
17-442	■ Erk 1/2 (Thr202/Tyr204)/(Thr185/Tyr187) Dual Detect CELISA Assay Kit (Fluorogenic Detection)
17-191	■ MAP Kinase/Erk Assay Kit, non-radioactive
MAB3054	■ Anti-Erk1/2, clone MK12
06-182	■ Anti-MAP Kinase 1/2 (Erk 1/2), CT
05-481	■ Anti-phospho-Erk 1/2 (Thr202/Tyr204, Thr185/Tyr187, clone 12D4
07-467	■ Anti-phospho-Erk 1/2 (Thr185/Tyr185)
16-111	■ Anti-MAPK Kinase 1/2 (Erk 1/2), agarose
05-957	■ Anti-MAP Kinase/Erk1, CT
05-157	■ Anti-MAP Kinase 2/ Erk2
06-333	■ Anti-Erk2
17-171	■ MAP Kinase/Erk Sampler pack
14-439	■ MAP Kinase 1/Erk1, active
14-515	■ MAP Kinase 1/Erk1, inactive
14-550	■ MAP Kinase 2/Erk2, active;
17-536	■ MAP Kinase 2/Erk2, inactive
17-218	■ Ras Assay Activation Assay Kit
05-1072	■ Anti-Ras (K-, H-, N-), clone 9A11.2

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit

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