abcam

Product datasheet

AKT/MAPK Signaling Pathway Antibody Cocktail ab151279

3 Abreviews 4 References 4 Images

Overview

Product name	AKT/MAPK Signaling Pathway Antibody Cocktail
Sample type	Cell culture extracts, Tissue Extracts, Cell Lysate, Human PBMC Lysate, Mouse muscle extract, Tissue Homogenate, Nuclear Extracts
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Product overview	ab151279 is a Western Blot Antibody Cocktail that contains 5 Rabbit antibodies targeting the phosphorylation status of 4 signaling proteins, AKT1 phospho S473, RPS6 phospho S235/236, ERK1/2 phospho Y204/187 and RKS1p90 phospho S380. Along with the total levels of 1 loading control protein, Rab11.
Notes	The cocktail targets downstream effectors of two important pro-survival pathways: the PI3K/PTEN/Akt/mTOR pathway and the Raf/MEK/ERK pathway, both of which are regulated by Ras. PI3K downstream effectors targeted in this cocktail are AKT1 phospho S473 and RPS6 phospho S235/236, whereas the downstream effectors of the MEK pathway are ERK1/2 phospho Y204/197 and p90RSK phospho S380. These two pathways are known for promoting cell growth, regulating apoptosis, chemotherapeutic drug resistance and cellular senescence. Both of these pathways affect protein translation by complex interactions regulating mTORC1/2 complexes; they regulate each other as well as other pathways such as Wnt/B-catenin, Jak/STAT, NF-κB and TGF-B.
Tested applications	**Suitable for:** WB

Properties

Storage instructions	Store at -20°C. Please refer to protocols.

Components	**200 µl**
250X AKT/MAPK Signaling Pathway Western Blot Cocktail	1 x 200µl

Relevance	Serine/threonine-protein kinase that acts downstream of ERK (MAPK1/ERK2 and MAPK3/ERK1) signaling and mediates mitogenic and stress-induced activation of the transcription factors CREB1, ETV1/ER81 and NR4A1/NUR77, regulates translation through RPS6 and EIF4B phosphorylation, and mediates cellular proliferation, survival, and differentiation

by modulating mTOR signaling and repressing pro-apoptotic function of BAD and DAPK1. In fibroblast, is required for EGF-stimulated phosphorylation of CREB1, which results in the subsequent transcriptional activation of several immediate-early genes. In response to mitogenic stimulation (EGF and PMA), phosphorylates and activates NR4A1/NUR77 and ETV1/ER81 transcription factors and the cofactor CREBBP. Upon insulin-derived signal, acts indirectly on the transcription regulation of several genes by phosphorylating GSK3B at 'Ser-9' and inhibiting its activity. Phosphorylates RPS6 in response to serum or EGF via an mTOR-independent mechanism and promotes translation initiation by facilitating assembly of the preinitiation complex. In response to insulin, phosphorylates EIF4B, enhancing EIF4B affinity for the EIF3 complex and stimulating cap-dependent translation. Is involved in the mTOR nutrient-sensing pathway by directly phosphorylating TSC2 at 'Ser-1798', which potently inhibits TSC2 ability to suppress mTOR signaling, and mediates phosphorylation of RPTOR, which regulates mTORC1 activity and may promote rapamycin-sensitive signaling independently of the PI3K/AKT pathway. Mediates cell survival by phosphorylating the pro-apoptotic proteins BAD and DAPK1 and suppressing their pro-apoptotic function. Promotes the survival of hepatic stellate cells by phosphorylating CEBPB in response to the hepatotoxin carbon tetrachloride (CCl4). Is involved in cell cycle regulation by phosphorylating the CDK inhibitor CDKN1B, which promotes CDKN1B association with 14-3-3 proteins and prevents its translocation to the nucleus and inhibition of G1 progression.

Cellular localization Cell Membrane, Cytoplasmic and Nuclear

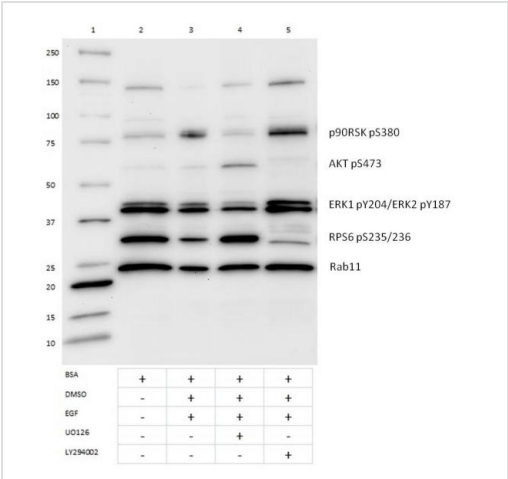
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab151279 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Suggested working concentration: 1/250

Images



Western Blot for AKT/MAPK Signaling Pathway Cocktail- Specificity (HRP)

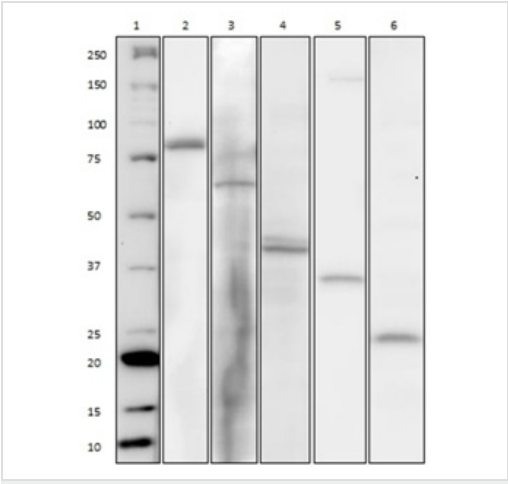
Performed under reducing conditions; Samples loaded at 30 µg/lane. Membrane blocking and secondary antibody incubation performed in 5% milk/PBST. Primary antibody incubation performed in 5% BSA/PBST.

Lane 1: Marker
Lane 2: A431 lysate starved
Lane 3: A431 lysate starved treated with 100 ng/mL of EGF for 30 min
Lane 4: A431 lysate starved and pretreated for 2 hours with 10µM U0126 (ab120241)
Lane 5: A431 lysate starved and pretreated for 2 hours with 30µM Ly294002 (ab120243)

Primary: AKT/MAPK signaling pathway antibody cocktail at 1/250 dilution

Secondary: HRP conjugated Goat anti-Rabbit secondary antibody at 1:10,000 dilution.

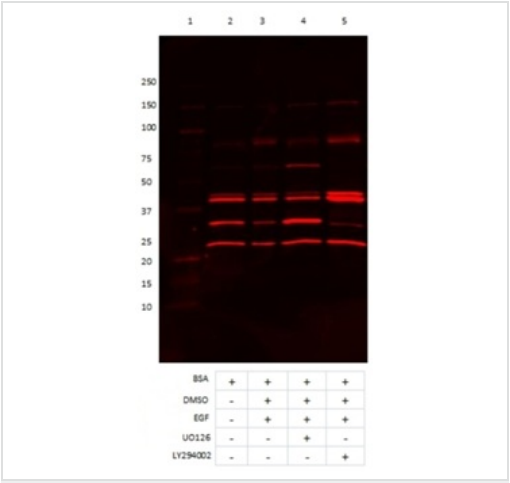
Observed bands:
RSK1p90 (pS380) band size: 83 kDa
AKT1 (pS473) band size: 56 kDa
ERK1 (pY204) and ERK2 (pY187) band size: 43 kDa
RPS6 (pS235/236) band size: 31 kDa
Rab11a band size: 24 kDa
Unknown band: 130kDa



Western Blot for AKT/MAPK Signaling Pathway Cocktail– Component Separation

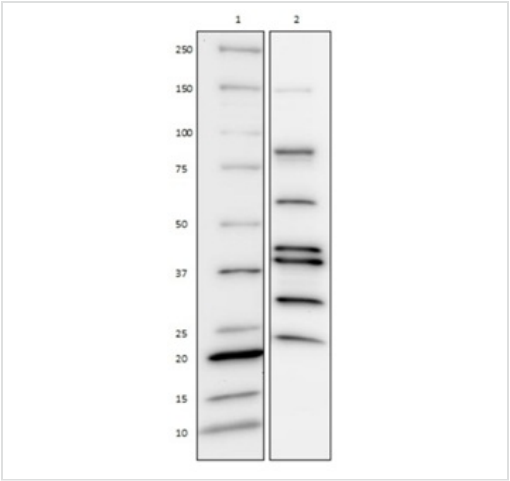
Developed using ECL; performed under reducing conditions; exposure time: 5 mins; samples run on a 4-20% gradient gel. Membrane blocking (2hrs RT) and secondary antibody incubation (2hrs RT) steps must be done in 5% milk, 1X PBS, 0.1% TWEEN-20.
Primary antibody incubation (24 hrs 4°C) must be done in 5% BSA, 1X PBS, 0.1% TWEEN-20. Secondary: HRP conjugated Goat anti-Rabbit secondary antibody at 1:10,000 dilution.
Samples:
Lane 1: Marker
Lanes 2-6: A431 cell lysate at 30 µg
Primary:
Lane 1: none
Lane 2: Anti-RSK1p90 (pS380)
Lane 3: Anti-AKT1 (pS473)
Lane 4: Anti-ERK1 (pY204) and ERK2 (pY187)

Lane 5: Anti-RPS6 (pS235/236)
Lane 6: Anti-Rab11a
Observed bands:
RSK1p90 (pS380) band size: 83 kDa
AKT1 (pS473) band size: 56 kDa
ERK1 (pY204) and ERK2 (pY187) band size: 43 kDa
RPS6 (pS235/236) band size: 31 kDa
Rab11a band size: 24 kDa



Western Blot for AKT/MAPK Signaling Pathway Cocktail- Specificity (IR)

All lysates loaded at 30 µg/lane.
Membrane blocking (2hrs RT) and secondary antibody incubation (2hrs RT) steps must be done in 5% milk, 1X PBS, 0.1% TWEEN-20.
Primary antibody incubation (24 hrs 4°C) must be done in 5% BSA, 1X PBS, 0.1% TWEEN-20, at 1/250 dilution.
Secondary: IR690 conjugated Goat anti-Rabbit secondary antibody at 1/10,000 dilution
Lane 1: Marker
Lane 2: A431 cells starved O/N
Lane 3: A431 cells starved O/N treated with 100 ng/mL of EGF for 30 min
Lane 4: A431 cells starved O/N and pretreated for 2 hours with 10 µM UO126 prior to EGF treatment
Lane 5: A431 cells starved O/N and pretreated for 2 hours with 30 µM Ly294002 prior to EGF treatment



Western Blot for AKT/MAPK Signaling Pathway Cocktail – Cross Reactivity

Developed using the ECL technique; performed under reducing conditions; exposure time: 5 mins; samples run on a 4-20% gradient gel.
Membrane blocking (2hrs RT) and secondary antibody incubation (2hrs RT) steps must be done in 5% milk, 1X PBS, 0.1% TWEEN-20.
Primary antibody incubation (24 hrs 4°C) must be done in 5% BSA, 1X PBS, 0.1% TWEEN-20.
Lane 1: Marker.
Lane 2: NIH3T3 cells starved overnight and treated with 50ng/mL of PDGF for 30 minutes lysate – 30 µg.
Primary: AKT/MAPK signaling pathway antibody cocktail at 1/250 dilution.
Secondary: HRP conjugated Goat anti-Rabbit secondary antibody at 1/10,000 dilution.
Observed bands:
RSK1p90 (pS380) band size: 83 kDa
AKT1 (pS473) band size: 56 kDa

ERK1 (pY204) and ERK2 (pY187) band size: 43 kDa

RPS6 (pS235/236) band size: 31 kDa

Rab11a band size: 24 kDa

Unknown band: 130kDa

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors