Datasheet for ABIN968835

anti-ERK1/2 antibody (pThr202)

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Overview

Quantity:	50 µg
Target:	ERK1/2 (MAPK1/3)
Binding Specificity:	pThr202
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This ERK1/2 antibody is un-conjugated
Application:	Western Blotting (WB), Intracellular Staining (ICS), BioImaging (BI)

Product Details

Immunogen:	Phosphorylated Rat ERK1 (T202/Y204) Peptide
Clone:	20A
Isotype:	IgG1
Cross-Reactivity:	Human, Mouse (Murine)
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. Please refer to us for technical protocols. 3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance. 4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive

Product Details

	deposits in plumbing. 5. Source of all serum proteins is from USDA inspected abattoirs located in the United States. 6. Triton is a trademark of the Dow Chemical Company.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	ERK1/2 (MAPK1/3)
Alternative Name:	ERK1/2 (MAPK1/3 Products)
Background:	The members of the Mitogen-Activated Protein Kinase (MAPK) family are components of a key signal transduction cascade that links events at the cell surface to responses in the nucleus. The signaling cascade is found in species as varied as yeast and humans, with many of the proteins being well conserved. In mammals the most widely studied members of the cascade are the Extracellular signal-Regulated Kinases, ERK1 (p44 MAPK) and ERK2 (p42 MAPK). ERK1 and ERK2 share 85% homology and are activated by extracellular signals such as growth factors, hormones, and phorbol esters. Activation occurs through a series of phosphorylations by kinases activating other kinases and eventually leading to phosphorylation of the ERKs. Growth factor stimulation leads to activation of Ras and Raf, leading to phosphorylation of MEK1 (MAPK/ERK kinase) which, in turn, activates the ERKs via dual phosphorylation. Once activated, the ERKs phosphorylate other cytoplasmic signalling molecules, cell-surface receptors, microtubule-associated proteins, and transcription factors in the nucleus. Thus, the active ERK has myriad downstream effectors that implicate it in the control of cell proliferation and differentiation, as well as regulation of the cytoskeleton. Furthermore, studies have shown that elevated ERK activity is associated with some cancers. The 20A monoclonal antibody recognizes the phosphorylated threonine 202 and tyrosine 204 (pT202/pY204) of human ERK1 and pT184/pY186 of human ERK2. The orthologous phosphorylation sites in murine ERK1 and ERK2 are T203/Y205 and T183/Y185. Synonyms: p44/42 MAPK, Extracellular signal-Regulated Kinase 1/2 (pT202/Y204)
Molecular Weight:	44/42 kDa

Application Details

Application Notes:	Bioimaging 1. Seed the cells in appropriate culture medium at ~10,000 cells per well in an 96-well Imaging

Application Details

	Plate and culture overnight. 2. Remove the culture medium from the wells, and fix the cells by adding 100 myl of Fixation Buffer to each well. Incubate for 10 minutes at room temperature (RT). 3. Remove the fixative from the wells, and permeabilize the cells using either 90% methanol, or Triton™ X-100: a. Add 100 myl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. OR b. Add 100 myl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT. 4. Remove the permeabilization buffer, and wash the wells twice with 100 myl of 1× PBS. 5. Remove the PBS, and block the cells by adding 100 myl of to each well. Incubate for 30 minutes at RT. 6. Remove the blocking buffer and add 50 myl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT. 7. Remove the primary antibody, and wash the wells three times with 100 myl of 1× PBS. 8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 myl to each well, and incubate in the dark for 1 hour at RT. 9. Remove the second step reagent, and wash the wells three times with 100 myl of 1× PBS. 10. Remove the PBS, and counter-stain the nuclei by adding 200 myl per well of 2 myg/ml Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 11. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN968534, ABIN968533, ABIN967700, ABIN967389
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	250 µg/mL
Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	-20 °C
Storage Comment:	Store undiluted at -20°C.

Publications

Product cited in:

Meng, Casey: "Activation of Gz attenuates Rap1-mediated differentiation of PC12 cells." in: **The Journal of biological chemistry**, Vol. 277, Issue 45, pp. 43417-24, (2002) (PubMed).

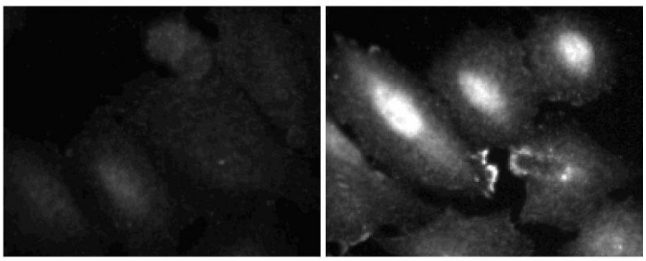
Sivaraman, Wang, Nuovo, Malbon: "Hyperexpression of mitogen-activated protein kinase in human breast cancer." in: **The Journal of clinical investigation**, Vol. 99, Issue 7, pp. 1478-83, (1997) (PubMed).

Clark, Hynes: "Ras activation is necessary for integrin-mediated activation of extracellular signal-regulated kinase 2 and cytosolic phospholipase A2 but not for cytoskeletal organization. " in: **The Journal of biological chemistry**, Vol. 271, Issue 25, pp. 14814-8, (1996) (PubMed).

Boulton, Cobb: "Identification of multiple extracellular signal-regulated kinases (ERKs) with antipeptide antibodies." in: **Cell regulation**, Vol. 2, Issue 5, pp. 357-71, (1991) (PubMed).

Images

Image 1. A431 (ATCC CRL-1555) cells were either left untreated (lane 1) or treated (lane 2) with 100 ng/ml EGF for 5 minutes at 37°C. The top panel was probed with ERK1/2 (ABIN967700) and the bottom was probed with ERK1/2 (pT202/pY204) (ABIN968835).



Immunofluorescence

Image 2. Immunofluorescent staining of A549 (ATCC CCL-185) cells. Cells were seeded in a 96 well imaging plate at ~10,000 cells per well. After overnight incubation, cells were either mock treated (media alone, left) or exposed to PMA (right) for 30 minutes. After treatment cells were stained using the alcohol perm protocol and the anti-ERK1/2 (pT202/pY204) antibody. The second step reagent was Alexa Fluor® 488 anti-mouse IgG. The image was taken on

a BD Pathway™ 855 Bioimager with a 20x objective. This antibody also stains HeLa (ATCC CCL-2) and U-2 OS (ATCC HTB-96) cells and worked with both the Triton™ X-100 and alcohol perm protocols.