Technical Data Sheet

Purified Mouse Anti-ERK3

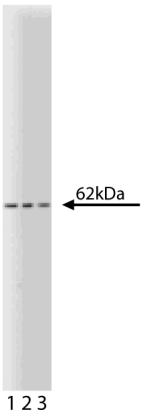
Product Information

Material Number:	**610105**
Size:	50 µg
Concentration:	250 µg/ml
Clone:	30/ERK3
Immunogen:	Rat ERK3 aa. 399-543
Isotype:	Mouse IgM
Reactivity:	QC Testing: Mouse Tested in Development: Human, Rat, Chicken, Dog
Target Molecular Weight:	62 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

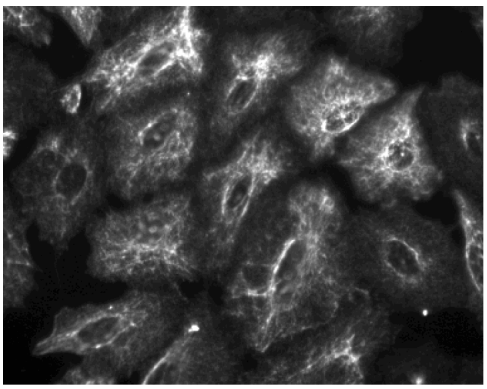
Description

The family of serine/threonine kinases known as ERKs (extracellular signal regulated kinases) or MAPKs (mitogen-activated protein kinases) are activated after cell stimulation by a variety of hormones and growth factors. Cell stimulation induces a signaling cascade that leads to phosphorylation of MEK (MAPK/ERK kinase) which, in turn, activates ERK via tyrosine and threonine phosphorylation. A myriad of proteins represent the downstream effectors for the active ERK and implicate it in the control of cell proliferation and differentiation, as well as regulation of the cytoskeleton. Activation of ERK is normally transient and cells possess dual specificity phosphatases that are responsible for its down-regulation. Furthermore, multiple studies have shown that elevated ERK activity is associated with some cancers. ERK3 is a 63kDa family member that is 43% homologous to ERK1 and ERK2. A 97kDa ERK3 isoform has also been described which possesses an additional 178 amino acids.

This antibody is routinely tested by Western blot analysis and immunofluorescent imaging. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Western blot analysis of ERK3 on a RSV-3T3 lysate. *Lane 1: 1:250, lane 2: 1:500, lane 3: 1:1000 dilution of the anti-ERK3 antibody.*



Immunofluorescent staining of A549 cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the methanol fix/perm protocol (see Recommended Assay Procedure) and the anti-ERK3 antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). Images were taken on a Pathway 855 imager using a 20x objective. This antibody also stained HeLa and U2OS cells and worked with both the Triton X100 and Methanol fix/perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20° C.

Application Notes

Application

Bioimaging	Routinely Tested
Western blot	Routinely Tested
Immunofluorescence	Tested During Development
Immunoprecipitation	Tested During Development

Recommended Assay Procedure:

Methanol Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 90% methanol. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Wash three times with PBS. Flick out PBS and add second step reagent. Incubate for 1 hour at RT. Wash three times with PBS. Image sample.

Triton-X 100 Procedure for a 96 well plate:

Remove media from wells. Add 100 µl/well fresh 3.7% Formaldehyde in PBS. Incubate for 10 minutes at room temperature (RT). Flick out and add 100 µl/well 0.1% Triton-X 100. Incubate for 5 minutes at RT. Flick out and wash twice with PBS. Flick out PBS and add 100 µl/well blocking buffer (3% FBS in PBS). Incubate for 30 minutes at RT. Flick out and add diluted antibody (diluted in blocking buffer). Incubate for 1 hour at RT. Flick out and wash three times with PBS. Flick out and add second step reagent. Incubate for 1 hour at RT. Flick out and wash three times with PBS. Image sample.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554002	HRP Goat Anti-Mouse Igs	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Igs	0.5 mg	Polyclonal

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. 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 deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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Cobb MH, Goldsmith EJ. How MAP kinases are regulated. *J Biol Chem.* 1995; 270(25):14843-14846.(Biology)

Milasincic DJ, Calera MR, Farmer SR, Pilch PF. Stimulation of C2C12 myoblast growth by basic fibroblast growth factor and insulin-like growth factor 1 can occur via mitogen-activated protein kinase-dependent and -independent pathways. *Mol Cell Biol.* 1996; 16(11):5964-5973.(Biology)

Sauma S, Friedman E. Increased expression of protein kinase C beta activates ERK3. *J Biol Chem.* 1996; 271(19):11422-11426.(Clone-specific: In vitro kinase assay)