Technical Data Sheet

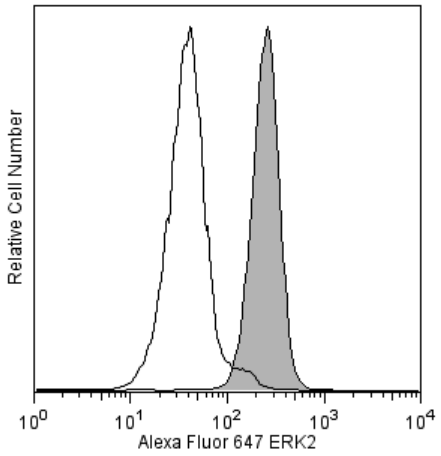
Alexa Fluor® 647 Mouse anti-ERK2

Product Information

Material Number:	**558526**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	G263-7
Immunogen:	Human ERK2 Synthetic Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	Human, Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The members of the *M*itogen-*A*ctivated *P*rotein *K*inase (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 *E*xtracellular signal-*R*egulated *K*inases, 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 (protein kinases and phosphatases), 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 G263-7 recognizes ERK2. It does not cross-react with ERK1. Clone G263-7 was originally characterized in human (A431) and mouse (NIH/3T3) cells.A human ERK2 synthetic peptide was used as immunogen.



Analysis of ERK2 in human epithelioid carcinoma. *HeLa S3 cells (ATCC CCL 2.2) were either transfected with ERK2 RNAi (open histogram) or untransfected (shaded histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-ERK2. Down-regulation of ERK2 expression is evident in the RNAi-transfected cells. Flow cytometry was performed on a BD FACSArray™ bioanalyzer system.*

Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)

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554655	Fixation Buffer	100 ml	(none)
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. 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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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