Technical Data Sheet

PE Mouse Anti-ERK1/2 (pT202/pY204)

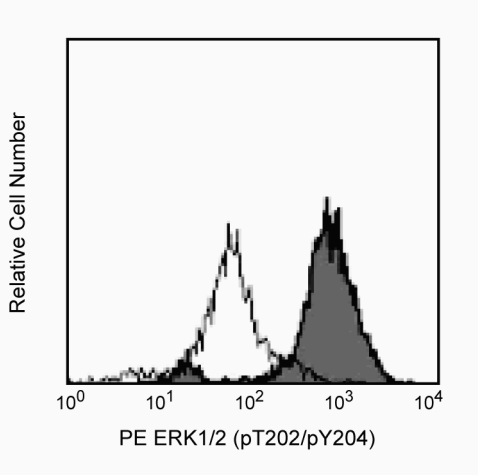
Product Information

Material Number:	**612566**
Alternate Name:	p44/42 MAPK; Extracellular signal-Regulated Kinase 1/2 (pT202/Y204)
Size:	50 tests
Vol. per Test:	20 µl
Clone:	20A
Immunogen:	Phosphorylated Rat ERK1 (T202/Y204) Peptide
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development by Western blot with the purified antibody (Cat. No. 612358): Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

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.



Flow cytometric analysis of ERK1/2 (pT202/pY204). *Human peripheral blood mononulcear cells (PBMC) were either left unstimulated (unshaded) or stimulated (shaded) with 40 nM PMA for 10 minutes at 37°C. Cells were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) for 10 minutes at 37°C and then permeabilized by adding BD Phosflow™ Perm Buffer III (Cat. No. 558050) for 30 minutes on ice. Cells were then washed twice in BD Pharmingen™ Stain Buffer (Cat. No. 554656) and stained with the PE mouse anti-ERK1/2 (pT202/pY204) antibody. Cells were analyzed on a BD FACSCalibur™ flow cytometry instrument. For intracellular staining of human whole blood, BD Phosflow™ Lyse/Fix buffer (Cat. No. 558049) may be used for fixation.*

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 with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

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Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

For more information about BD Phosflow™: Please refer to http://www.bdbiosciences.com/support/resources/phosflow/index.jsp

Investigators may also find the following protocols to be helpful:

Phosflow protocol for human PBMC: Please refer to http://www.bdbiosciences.com/documents/Phosflow_Protocol_for_Human_PBMCs.pdf
Phosflow protocol for human whole blood: Please refer to
http://www.bdbiosciences.com/documents/Phosflow_Protocol_for_Human_Whole_Blood_Samples.pdf
Phosflow protocol for adherent cells: Please refer to http://www.bdbiosciences.com/support/resources/protocols/protocol_adherent.jsp

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
557870	Fix Buffer I	250 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
612358	Purified Mouse Anti-ERK1/2 (pT202/pY204)	50 µg	20A

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. 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.
3. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
4. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Boulton TG, Cobb MH. Identification of multiple extracellular signal-regulated kinases (ERKs) with antipeptide antibodies. *Cell Regul.* 1991; 2(5):357-371. (Biology)
Clark EA, Hynes RO. Ras activation is necessary for integrin-mediated activation of extracellular signal-regulated kinase 2 and cytosolic phospholipase A2 but not for cytoskeletal organization. *J Biol Chem.* 1996; 271(25):14814-14818. (Biology)
Sivaraman VS, Wang H, Nuovo GJ, Malbon CC. Hyperexpression of mitogen-activated protein kinase in human breast cancer. *J Clin Invest.* 1997; 99(7):1478-1483. (Biology)

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