Technical Data Sheet

Pacific Blue™ Mouse anti-ERK1/2 (pT202/pY204)

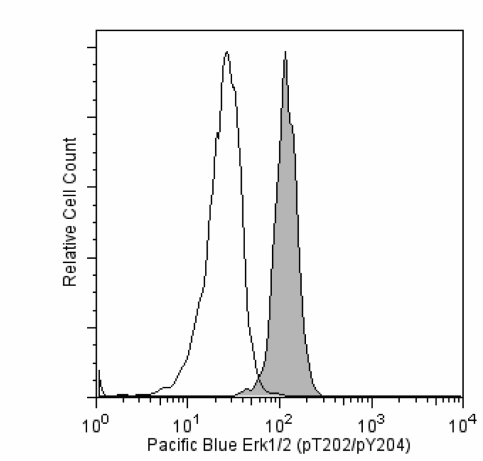
Product Information

Material Number:	**560314**
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: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA, protein stabilizer, 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.



Analysis of Erk1/2 (pT202/pY204) in human peripheral blood lymphocytes. *Human peripheral blood mononuclear cells (PBMC) were either left untreated (unshaded) or treated (shaded) with 400 nM phorbol 12-myristate 13-acetate (PMA) (Sigma, Cat. No. P8139) for 15 minutes at 37°C. The PBMC were fixed with BD Cytofix™ buffer (Cat. No. 554655) for 10 minutes at 37°C, permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes and were then stained with Pacific Blue™ Mouse anti-ERK1/2 (pT202/pY204). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD FACSCanto™ II flow cytometry 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 is conjugated to Pacific Blue™ under optimum conditions, and unreacted Pacific Blue™ was removed.

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The purified or conjugated mAb was characterized by flow cytometry (Flow) and western blot (WB) using these model systems:

Method	*Species*	*Cells*	*Treatment*	*Fixation*	*Perm buffer*	*Result*
Flow	Human	PBMC	PMA	Cytofix	Perm I, II, or III	Upregulated expression
		Whole Blood	PMA	Lyse/Fix	Perm I, II, or III	Upregulated expression
WB	Human	A-431	EGF			44/42-kDa band induced

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

This antibody conjugate is suitable for intracellular staining of human whole blood (using BD Phosflow™ Lyse/Fix Buffer) and peripheral blood mononuclear cells (using BD Cytofix™ Fixation Buffer). Any of the three BD Phosflow™ permeabilization buffers may be used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 mL	(none)
554655	Fixation Buffer	100 mL	(none)
557885	Perm/Wash Buffer I	125 mL	(none)
558052	Perm Buffer II	125 mL	(none)
558050	Perm Buffer III	125 mL	(none)
612358	Purified Mouse Anti-ERK1/2 (pT202/pY204)	50 µg	20A
612359	Purified Mouse Anti-ERK1/2 (pT202/pY204)	150 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Pacific Blue™ has a maximum absorption of 416 nm and maximum emission of 451 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
4. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
7. 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.
8. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
9. Species testing during development may have been performed with a different format of the same clone. Selected applications have been tested for cross-reactivity.

References

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Irish JM, Czerwinski DK, Nolan GP, Levy R. Altered B-cell receptor signaling kinetics distinguish human follicular lymphoma B cells from tumor-infiltrating nonmalignant B cells. *Blood.* 2006; 108(9):3135-3142. (Clone-specific: Flow cytometry)

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Kim SC, Hahn JS, Min YH, Yoo NC, Ko YW, Lee WJ. Constitutive activation of extracellular signal-regulated kinase in human acute leukemias: combined role of activation of MEK, hyperexpression of extracellular signal-regulated kinase, and downregulation of a phosphatase, PAC1. *Blood.* 1999; 93(11):3893-3899. (Clone-specific: In vitro kinase assay, Western blot)

Krutzik PO, Nolan GP. Intracellular phospho-protein staining techniques for flow cytometry: monitoring single cell signaling events. *Cytometry A.* 2003; 55(2):61-70. (Clone-specific: Flow cytometry)

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