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 $\leq 0.09\%$ 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.

BD Biosciences

bdbiosciences.	com				
United States 877.232.8995	Canada 800.979.9408	Europe 32.53.720.550	Japan 0120.8555.90	Asia Pacific 65.6861.0633	Latin America/Caribbean 55.11.5185.9995
For country cor	ntact informatio	on, visit bdbiosci	ences.com/conta	ct	
Conditions: The in of any patents. BL use of our product product or as a co written authoriza For Research Use of Unless otherwise	formation disclose) Biosciences will n ts. Purchase does n mponent of anoth tion of Becton, Dic Only. Not for use ir noted, BD, BD Loge	d herein is not to b ot be help responsi ot include or carry a er product. Any use kinson and Compar o diagnostic or there o and all other trade	e constructed as a re ble for patent infrin any right to resell ou e of this product oth ny is stictly prohibite apeutic procedures. emarks are property	ecommendation to u gement or other vio r transfer this produ er than the permitte d. Not for resale. v of Becton, Dickinso	ise the above product in violation lations that may occur with the ct either as a stand-alone ed use without the express n and Company. © 2011 BD





Application Notes

Application

Intracellular	staining	(flow	evtometry	ī
muucomunu	stanning	110 **	cytometry	<i>.</i>

Recommended Assay Procedure:

For more information about BD PhosflowTM: 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/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	

Routinely Tested

Product Notices

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 1. 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.

- Source of all serum proteins is from USDA inspected abattoirs located in the United States. 3.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at 4. 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)

BD Biosciences

bdbiosciences.com United States Canada Europe 877.232.8995

800.979.9408 32.53.720.550 0120.8555.90 For country contact information, visit bdbiosciences.com/contact

Latin America/Caribbean 65.6861.0633 55.11.5185.9995

Conditions: The information disclosed herein is not to be constructed as a recommendation to use the above product in violation of any patents. BD Biosciences will not be help responsible for patent infringement or other violations that may occur with the use of our products. Buckness will not be help responsible for patent infringement or other violations that may occur with the product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Becton, Dickinson and Company is stictly prohibited. For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale. Unless otherwise noted, BD, BD Logo and all other trademarks are property of Becton, Dickinson and Company. © 2011 BD

Asia Pacific

Japan

