

## Technical Data Sheet

**BV421 Mouse Anti-ERK1/2 (pT202/pY204)****Product Information**

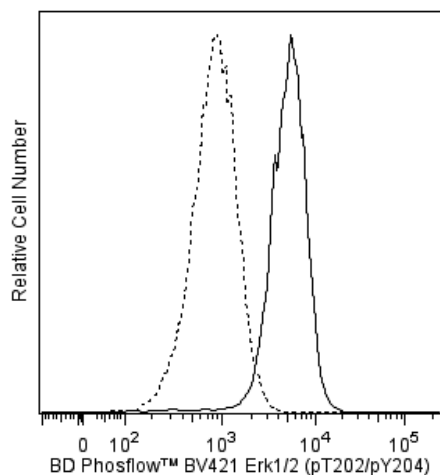
<b>Material Number:</b>	<b>562981</b>
<b>Alternate Name:</b>	p44/42 MAPK; Extracellular signal-Regulated Kinase 1/2 (pT202/Y204)
<b>Size:</b>	50 tests
<b>Vol. per Test:</b>	5 µl
<b>Clone:</b>	20A
<b>Immunogen:</b>	Phosphorylated Rat ERK1 (T202/Y204) Peptide
<b>Isotype:</b>	Mouse IgG1
<b>Reactivity:</b>	QC Testing: Human Tested in Development: Mouse, Rat
<b>Storage Buffer:</b>	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.

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon™ BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue™ conjugates.



**Flow cytometric analysis of Erk1/2 (pT202/pY204) expression in stimulated human peripheral blood lymphocytes.** Whole blood was either left untreated (dashed line histogram) or treated (solid line histogram) with 400 nM Phorbol 12-Myristate 13-Acetate (PMA; Sigma, Cat. No. P8139) for 15 minutes at 37°C. Erythrocytes were lysed and the leucocytes were fixed with 1X BD Phosflow™ Lyse/Fix Buffer (Cat. No. 558049) for 10 minutes at 37°C. The cells were then permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for 30 minutes and stained with BD Phosflow™ BV421 Mouse Anti-Erk1/2 (pT202/pY204) (Cat. No. 562981). The fluorescence histograms were derived from gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD LSRFortessa™ Cell Analyzer System.

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## 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 BD Horizon™ BV421 under optimum conditions, and unconjugated antibody and free BD Horizon™ BV421 were removed.

## Application Notes

### Application

Intracellular staining (flow cytometry)	Routinely Tested
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## Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
562438	BV421 Mouse IgG1, k Isotype Control	50 µg	X40

## Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100-µl experimental sample (a test).
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. An isotype control should be used at the same concentration as the antibody of interest.
4. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.
5. 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.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
7. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
8. Brilliant Violet™ 421 is a trademark of Sirigen.
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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