Technical Data Sheet

PE Mouse anti-Elk-1 (pS383)

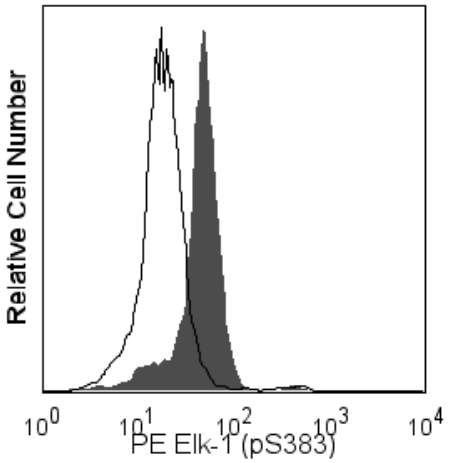
Product Information

Material Number:	**560412**
Alternate Name:	ELK1
Size:	50 tests
Vol. per Test:	20 µl
Clone:	M21-1721
Immunogen:	Phosphorylated Human Elk-1 Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Predicted due to immunogen sequence identity: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

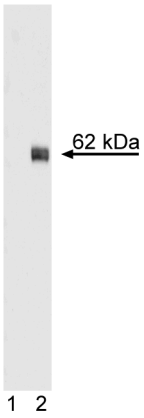
Description

Elk-1 is a 62-kDa member of the Ets oncogene family of transcription factors and a member of the subfamily of ternary complex factors (TCF). Ets proteins mediate a variety of gene activities in response to serum and growth factors. Proteins in the TCF subfamily form a ternary complex by binding to the Serum Response Element (SRE) in conjunction with a dimer of Serum Response Factors (SRF). Elk-1 is phosphorylated by mitogen-activated protein (MAP) kinase pathways in vivo at a cluster of S/T motifs at its carboxy-terminus. Phosphorylation at these sites, particularly at serine 383 (S383), is critical for Elk-1 transcriptional activation. Studies have shown that Elk-1 is a direct target of activated MAP kinase, and that it is also a target of the stress-activated kinase SAPK/JNK.

The M21-1721 monoclonal antibody recognizes the phosphorylated S383 of activated Elk-1. The orthologous phosphorylation sites of mouse and rat Elk-1 are S384 and S382, respectively.



Analysis Elk-1 (pS383) in activated human peripheral blood mononuclear cells (PBMC). *PBMC were isolated by density gradient centrifugation (Ficoll-Paque™ PLUS, Cat. No. 17-1440-02), and cultured with 20 µg/ml PHA-P (Sigma-Aldrich, Cat. No. L1668) for 3 days, then either left untreated (open histogram) or treated with PMA at 50 nM/10^6 cells for 15 minutes (shaded histogram; Sigma-Aldrich, Cat. No. P8139). Cells were then fixed in BD Cytofix™ Fixation Buffer (Cat. No. 554655) at 37°C for 10 minutes, then permeabilized with BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for at least 30 minutes, and then stained with PE Mouse anti-Elk-1 (pS383). Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*



Western blot analysis of Elk-1 (pS383). *The specificity of mAb M21-1721 was confirmed by western blot analysis using unconjugated Mouse anti-Elk-1 (pS383) antibody on lysates from PBMC that were cultured with PHA for 3 days (lane 1) or PBMC that were cultured with PHA for 3 days and then treated with PMA for 15 minutes (lane 2). Elk-1 (pS383) is identified as a band of 62 kDa in the PMA-treated cells.*

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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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	PHA-stimulated PBMC	PMA	Cytofix	Perm I, II, or III	Upregulated expression
WB	Human	PHA-stimulated PBMC	PMA			62-kDa band induced
		HeLa S3	PMA			62-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 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
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)

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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Bardwell AJ, Abdollahi M, Bardwell L. Docking sites on mitogen-activated protein kinase (MAPK) kinases, MAPK phosphatases and the Elk-1 transcription factor compete for MAPK binding and are crucial for enzymic activity. *Biochem J.* 2003; 370:1077-1085. (Biology)

Cavigelli M, Dolfi F, Claret FX, Karin M. Induction of c-fos expression through JNK-mediated TCF/Elk-1 phosphorylation. *EMBO J.* 1995; 14(23):5957-5964. (Biology)

Espanel X, Wälchli S, Rückle T et al. Mapping of synergistic components of weakly interacting protein-protein motifs using arrays of paired peptides. *J Biol Chem.* 2003; 278(17):15162-15167. (Biology)

Sugimoto T, Stewart S, Guan KL. The calcium/calmodulin-dependent protein phosphatase calcineurin is the major Elk-1 phosphatase. *J Biol Chem.* 1997; 272(47):29415-29418. (Biology)

Tian J, Karin M. Stimulation of Elk1 transcriptional activity by mitogen-activated protein kinases is negatively regulated by protein phosphatase 2B (calcineurin). *J Biol Chem.* 1999; 274(21):15173-15180. (Biology)

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