

Technical Data Sheet

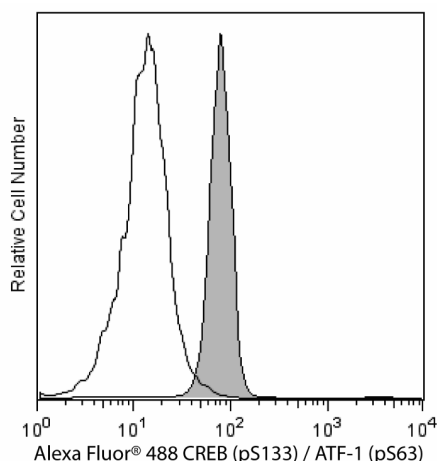
Alexa Fluor® 488 Mouse Anti-CREB (pS133) / ATF-1 (pS63)**Product Information**

Material Number:	558435
Size:	50 tests
Vol. per Test:	20 µl
Clone:	J151-21
Immunogen:	Phosphorylated Human CREB Peptide
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human Predicted: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Transcription of various genes is regulated by the cyclic AMP (cAMP) signal transduction pathway through a family of cAMP Responsive Element (CRE)-Binding transcription factors that include CREB, CREM (CRE Modulator), and ATF-1 (Activating Transcription Factor 1). The genes for these transcription factors encode multiple isoforms that are created by alternative splicing, alternative initiation codons, and alternative intronic promoters. Homo- and heterodimers of these members of the basic domain-and-leucine zipper superfamily of proteins bind the palindromic TGACGTCA sequence of CRE. CREB is expressed in all somatic cells, and many different stimuli activate CREB by phosphorylation of its serine 133 (S133). The transcriptional activity and specificity of CREB are regulated by the signaling pathways initiated by the various stimuli. Transcriptional regulation by CREB family members has been implicated in many physiological responses to extracellular and environmental stimuli, such as memory, tissue growth and development, and homeostasis.

The J151-21 monoclonal antibody recognizes the phosphorylated S133 of human CREB in addition to cross-reacting with S63 of human ATF-1.



Analysis of CREB (pS133) / ATF-1 (pS63) in human peripheral blood lymphocytes. Human whole blood was either stimulated with PMA and Ionomycin for 15 minutes at 37°C (shaded histogram) or unstimulated (open histogram). The cells were lysed and fixed with 1X BD™ Phosflow Lyse/Fix Buffer (Cat. No. 558049) for 10-15 minutes at 37°C, then permeabilized (BD™ Phosflow Perm Buffer II, Cat. No. 558052) on ice for 30 minutes, and then stained with the Alexa Fluor® 488 mouse anti-CREB (pS133) / ATF-1 (pS63) antibody. For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD™ FACSCalibur 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 was conjugated to Alexa Fluor® 488 under optimum conditions, and unreacted Alexa Fluor® 488 was removed.

Application Notes**Application**

Intracellular staining (flow cytometry)	Routinely Tested
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Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558052	Perm Buffer II	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)

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558050	Perm Buffer III	125 ml	(none)
557885	Perm/Wash Buffer I	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- μ l experimental sample (a test).
2. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.
3. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
4. 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.
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.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
8. Please refer to www.bdbiosciences.com/pharming/en/protocols for technical protocols.

References

Shaywitz AJ, Greenberg ME. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem.* 1999; 68:821-861.(Biology)