Technical Data Sheet

PE Mouse anti-TBK1

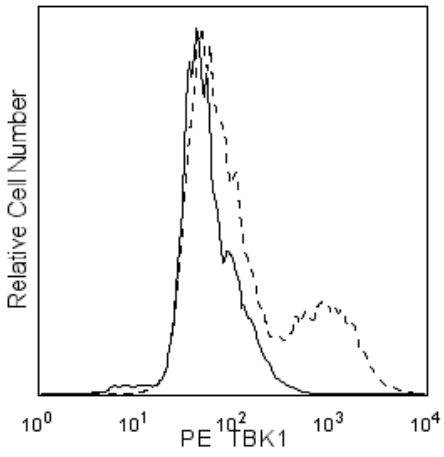
Product Information

Material Number:	**558696**
Alternate Name:	T2K, NAK
Size:	50 tests
Vol. per Test:	20 µl
Clone:	637Ig11.2
Immunogen:	Human TBK1 C terminus Recombinant Protein
Isotype:	Mouse IgG1, κ
Reactivity:	Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

NF-κB is a ubiquitously expressed transcription factor that regulates many cytokine and Ig genes. It is involved in immune, inflammatory, viral, and acute phase responses. In most cells, NF-κB is sequestered in an inactive cytoplasmic form via interactions with the inhibitory proteins IκBα, IκBβ, and IκBε. Stimulation induces the release, activation, and nuclear translocation of NF-κB. Release of NF-κB results from the phosphorylation and proteolytic degradation of the IκB proteins. Two cytokine-inducible IκB kinases (IKKα and IKKβ) phosphorylate and target the IκB proteins for degradation via the ubiquitin pathway. IKKγ/NEMO, a third member of the IKK complex, functions as a regulatory subunit and interacts directly with IKKβ. TBK1 (TANK-binding kinase 1, also known as T2K or NAK), a protein of 729 amino acids, is another member of the IKK family of kinases regulating NF-κB downstream of the tumor necrosis factor and Toll-like receptor pathways. TBK1 forms a complex with the adaptor proteins TANK (TRAF-associated NF-κB activator) and TRAF2 (TNF-receptor-associated factor 2), and this oligomer is required for activation and phosphorylation of TBK1 at serine 172.

The 637Ig11.2 monoclonal antibody recognizes human TBK1, regardless of phosphorylation status. Our in-house testing is performed on a cell line that has been co-transfected with TBK1 and IRF-7 because we have been unable to detect endogenous TBK1 by western blotting of lysates from activated cell lines (HeLa activated with PMA and A431 activated with PDGF). In the transfectants, the over-expression of TBK1 is necessary for phosphorylation of IRF-7, but the presence of IRF-7 does not affect TBK1 expression or phosphorylation. We confirmed that mAb 637Ig11.2 does not cross-react with IRF-7 by western blot analysis using the purified antibody.



Analysis of TBK1 in transfected human epithelial cells. *LEFT: The 293 fetal kidney cell line was either co-transfected with TBK1 and IRF-7 expression vectors (dashed histogram) or un-transfected (solid line). After 24 hours, the cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with PE Mouse anti-TBK1. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system. RIGHT: The specificity of mAb 637Ig11.2 was confirmed by western blot using unconjugated antibody on lysates from untransfected (left panel) and TBK1/IRF-7 co-transfected (right panel) 293 cells. The TBK1-Myc tagged fusion protein is identified as a band of 84 kDa in the lysate of co-transfected cells.*

Preparation and Storage

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.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
558050	Perm Buffer III	125 ml	(none)
554655	Fixation Buffer	100 ml	(none)
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. 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).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Kishore N, Huynh QK, Mathialagan S, et al. IKK-i and TBK-1 are enzymatically distinct from the homologous enzyme IKK-2: comparative analysis of recombinant human IKK-i, TBK-1, and IKK-2.. *J Biol Chem.* 2002; 277(16):13840-13847. (Biology)

Viatour P, Merville M-P, Bours V, Chariot A. Phosphorylation of NF-kappaB and IkappaB proteins: implications in cancer and inflammation. *Trends Biochem Sci.* 2005; 30(1):43-52. (Biology)