Technical Data Sheet

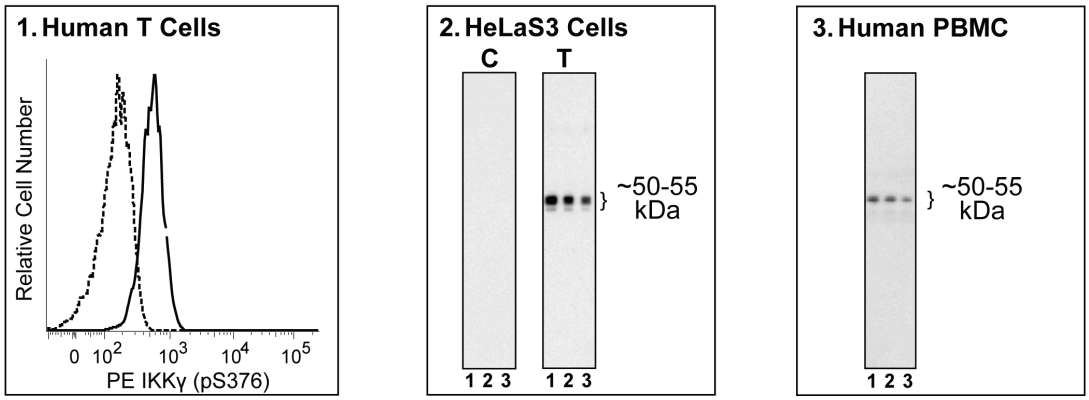
PE Mouse anti-Human IKKγ (pS376)

Product Information

Material Number:	**562590**
Alternate Name:	IKBKG; IKKγ; IKKG; IKK-gamma; AMCBX1; FIP3; IKKAP1; IP1; IP2; IPD2; NEMO
Size:	50 tests
Vol. per Test:	5 µl
Clone:	N19-39
Immunogen:	Phosphorylated Human IKKγ Peptide
Isotype:	Mouse IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The N19-39 monoclonal antibody specifically binds to the IKK-gamma protein phosphorylated at the Ser376 site, ie, IKKγ (pS376). IKKγ is also known an NEMO (NF-kappa-B essential modifier) and is encoded by the *IKBKG* (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma) gene. The nuclear transcription factor kappa-B, NF-κB, is controlled by interaction with an inhibitory subunit, IκB, which restricts NF-κB to the cytoplasm. Following stimulation by various cytokines or other stimuli, IκB becomes degraded and NF-κB is released to the nucleus. The release of IκB from NF-κB is thought to be a critical point in the activation of NF-κB signal pathways. Activated NF-κB regulates genes involved in various pathways including inflammation, immunity, and cell survival signaling pathways. A group of proteins form an NF-κB regulatory complex, or signalsome. Two members of this complex are a pair of closely related serine/threonine kinases, IKKα and IKKβ (also called IKK-1 and IKK-2), which phosphorylate critical residues of IκB, thus targeting it for subsequent degradation. The IKK complex contains similar amounts of IKKα, IKKβ as well as two other polypeptides, which are differentially processed forms of a third subunit, IKKγ. IKKα and IKKβ become activated following phosphorylation by upstream kinases, including NF-κB-inducing kinase (NIK)4 and MEKK1. IKKβ has been shown to phosphorylate IKKγ at serine 376 in response to signaling through the TNF receptor or the Tax oncoprotein of human T-cell leukemia virus type 1. IKKγ has an essential regulatory role in the IKK complex and is required for the activation of the NF-κB pathway in response to multiple cellular stimuli. IKKγ isoforms migrate as a doublet of ~50-55 kDa upon Western blot analysis.



Analyses of IKKγ (pS376) expression.

Panel 1: *Multicolor flow cytometric analysis of IKKγ (pS376) expression by human T lymphocytes. Human whole blood (collected with heparin) was either left unstimulated (dashed line histogram) or stimulated (solid line histogram) with 400 nM PMA and 250 ng/ml Ionomycin (Sigma, Cat No. I-0634) at 37°C for 10 minutes. Cells were fixed in 1X BD Phosflow Lyse/Fix Buffer (Cat. No. 558049 for 5X stock) at 37°C for 10 min. The cells were permeabilized in BD Phosflow™ Perm Buffer III (Cat. No. 558050) on ice for at least 30 min (or overnight at -20°C), washed and then stained with BD Phosflow™ PE Mouse anti-Human IKKγ (pS376) (Cat. No. 562590) and APC Mouse Anti-Human CD3 (Cat. No. 561810/555335/561811) antibodies. The fluorescence histograms were derived from CD3 positive-gated events with the forward and side light-scatter characteristics of intact lymphocytes. Flow cytometry was performed using a BD FACSCanto™ II Flow Cytometer System.*

Panel 2: *Western blot analysis of IKKγ (pS376) in human epithelioid carcinoma. HeLa S3 cells (ATCC CCL 2.2) were cultured overnight in medium containing 0.1% FBS and then either not treated (C) or stimulated (T) with recombinant human Tumor Necrosis Factor (Cat. No. 554618; 25 ng/ml) and Calyculin A (Calbiochem Cat. No. 208851; 50 nM) at 37°C for 10 minutes. Lysates (15 µg total cell protein/lane) were blotted using Purified Mouse Anti-Human IKKγ (pS376) antibody (Cat. No. 562589; at 1.0, 0.5 and 0.25 µg/ml for Lanes 1, 2, 3, respectively), HRP Goat Anti-Mouse Ig (Cat. No. 554002) and a chemiluminescent detection system. Phosphorylated IKKγ proteins were identified as ~50-55 kDa bands by blotting.*

Panel 3: *Western blot analysis of IKKγ (pS376) expressed by human peripheral blood mononuclear cells (PBMC). Human PBMC were stimulated with 50 nM Phorbol 12-Myristate 13-Acetate (PMA, Sigma, Cat. No. P-8139) at 37°C for 10 minutes, and lysates were analyzed by Western blotting as described above.*

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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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

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	Whole blood	PMA/Ionomycin	Lyse/Fix	Perm III	Induced
	Human	PBMC	PMA	Cytofix	Perm III	Induced
	Human	PBMC	TNF +/– Calyculin A	Cytofix	Perm III	Induced. Best induction with TNF + Calyculin A; some induction with Calyculin A alone but not with TNF alone.
	Human	HeLa S3 (serum-starved)	TNF + Calyculin A + peptide blocking	Cytofix	Perm I	Induced. Blocked by pS376 phospho peptide but not by non-phospho peptide.
WB	Human	PBMC	PMA			50-kDa band induced
	Human	PBMC	TNF +/– Calyculin A			50-kDa band induced. Best induction with TNF + Calyculin A; some induction with Calyculin A alone but not with TNF alone.
	Human	HeLa S3 (serum-starved)	TNF + Calyculin A + peptide blocking			50-kDa band induced. Blocked by pS376 phospho peptide but not by non-phospho peptide.

Suggested Companion Products

Catalog Number	Name	Size	Clone
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
561810	APC Mouse Anti-Human CD3	25 tests	UCHT1
555335	APC Mouse Anti-Human CD3	100 tests	UCHT1
561811	APC Mouse Anti-Human CD3	500 tests	UCHT1
562589	Purified Mouse anti-Human IKKγ (pS376)	50 µg	N19-39

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. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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

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