Technical Data Sheet

PE Mouse anti-IRF-7

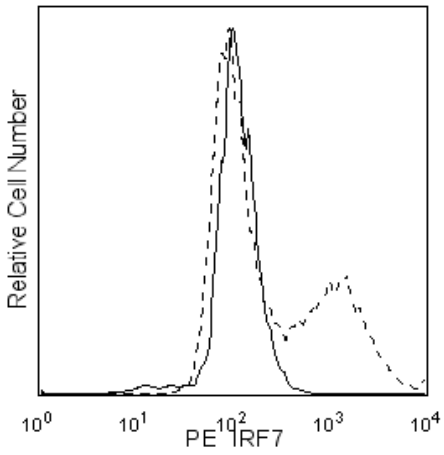
Product Information

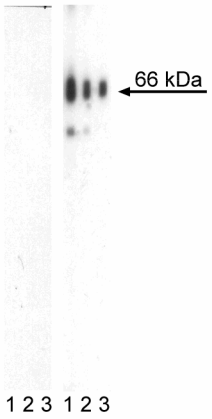
Material Number:	**558706**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	K40-321
Immunogen:	Human IRF-7 Recombinant Protein
Isotype:	Mouse (BALB/c) IgG1, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Interferon regulatory factor 7 (IRF-7) is a transcription factor that regulates anti-viral defenses by controlling the induction of type-I interferon (IFN) responses. IRF-7 expression is induced in lymphoid cells by virus infection, as well as by IFN, lipopolysaccharide, and TNF-α. IRF-7 responses are initiated by Toll-like receptors (TLR) or the cytoplasmic protein retinoic acid inducible gene I (RIG-I). Upon TLR activation, it forms cytoplasmic complexes with MyD88, an adaptor in the TLR signaling pathways. The TLR-dependent and RIG-I-dependent pathways activate kinases, such as IKK-ε and TBK1, that phosphorylate IRF-7 and induce movement of IRF-7-containing complexes to the nucleus, where it preferentially activates IFN-α promoters.

The K40-321 monoclonal antibody recognizes human IRF-7, regardless of phosphorylation status. Our in-house testing is performed on a cell line that has been co-transfected with TBK1 and IRF-7. Phosphorylation of IRF-7 in the transfectants requires virus infection or over-expression of a signaling molecule of the RIG-I pathway, such as TBK1. Phosphorylation of endogenous IRF-7 in untransfected cells has not yet been detected. We confirmed that mAb K40-321 does not cross-react with TBK1 by Western blot analysis using the purified antibody.





Analysis of IRF-7 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-IRF-7. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system. RIGHT: The specificity of mAb K40-321 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 transfected IRF-7 protein is identified as a band of 66 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.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

BD Biosciences

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Suggested Companion Products

Catalog Number	Name	Size	Clone
559320	PE Mouse IgG1, κ Isotype Control	100 tests	MOPC-21
554655	Fixation Buffer	100 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 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

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Hoshino K, Sugiyama T, Matsumoto M, et al. IκB kinase-α is critical for interferon-α production induced by Toll-like receptors 7 and 9. *Nature.* 2006; 440(7086):949-953. (Biology)

Kawai T, Sato S, Ishii KJ, et al. Interferon-α induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat Immunol.* 2004; 5(10):1061-1068. (Biology)

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Matikainen S, Sirén J, Tissari J, et al. Tumor necrosis factor alpha enhances influenza A virus-induced expression of antiviral cytokines by activating RIG-I gene expression. *J Virol.* 2006; 80(7):3515-3522. (Biology)

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