Technical Data Sheet

Alexa Fluor® 488 Mouse anti-IRF-7 (pS477/pS479)

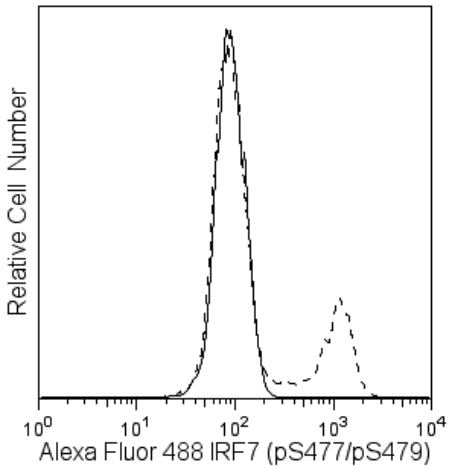
Product Information

Material Number:	**558629**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	K47-671
Immunogen:	Phosphorylated Human IRF-7 Peptide
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 K47-671 monoclonal antibody recognizes human IRF-7 phosphorylated at serines 477 and 479 (pS477/pS479). 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 K47-671 does not cross-react with TBK1 by Western blot analysis using the purified antibody.



Analysis of IRF-7 (pS477/pS479) in transfected human epithelial cells. *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 Alexa Fluor® 488 Mouse anti-IRF-7 (pS477/pS479) (Cat. No. 558629). 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

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

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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. Alexa Fluor® 488 fluorochrome emission is collected at the same instrument settings as for fluorescein isothiocyanate (FITC).
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
8. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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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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