Technical Data Sheet

Alexa Fluor® 647 Mouse anti-Human IRAK4

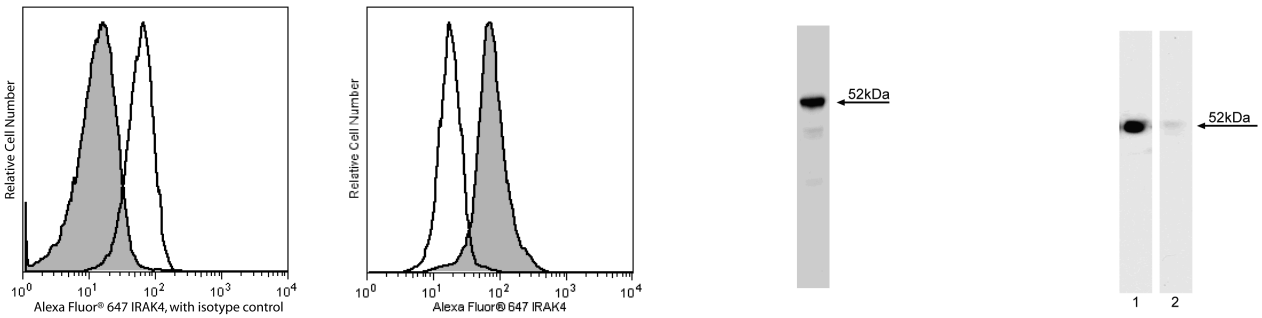
Product Information

Material Number:	**560315**
Alternate Name:	IRAK-4, REN64, Q69FE3
Size:	50 tests
Vol. per Test:	20 µl
Clone:	L29-525
Immunogen:	Human IRAK4 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

Interleukin 1 (IL-1) is a proinflammatory cytokine that mediates the host inflammatory response. IL-1 exerts its biological effects through its type I receptor (IL-1RI), which is found on most cell types. When IL-1 binds to the IL-1RI, NF-κB (transcription factor) is activated and translocates to the nucleus where it induces gene expression. **I**L-1-**r**eceptor-**a**ssociated **k**inase (IRAK) has been reported to co-immunoprecipitate with IL-1RI. The IRAK family is made up of four identified Pelle homologs that regulate the activation of NF-κB and **m**itogen-**a**ctivated **p**rotein (MAP) kinase pathways. IRAK4 is a unique member of the IRAK family because it is the closest mammalian homolog to Pelle. Also, when overexpressed, its kinase activity allows it to activate both the NF-κB and mitogen-activated protein (MAP) kinase pathways, and block the IL-1-induced activation and modification of IRAK1. IRAK4 is also able to phosphorylate IRAK1, which suggests that IRAK4 affects the early signal transduction of Toll/IL-1 receptors before the involvement of IRAK1.

The L29-525 monoclonal antibody recognizes human IRAK4, regardless of phosphorylation status. The specificity of this antibody conjugate for flow cytometric analysis was validated by confirming that RNA-mediated interference (RNAi) of the specific protein reduced the staining of the cells (see figure). Furthermore, the capacity of the RNAi to down-regulate the expression of the relevant protein was confirmed by western blot analysis.



Analysis of IRAK4 in activated human peripheral blood lymphocytes (far left panel). *Human peripheral blood mononuclear cells (PBMC) were lysed and fixed with 1X BD™ Phosflow Lyse/Fix Buffer (Cat. No. 558049) for 10 minutes at 37ºC, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with either Alexa Fluor® 647 Mouse IgG1 κ Isotype control (Cat. No. 557783, shaded histogram) or Alexa Fluor® 647 Mouse anti-Human IRAK4 (open histogram). For data analysis, lymphocytes were selected by scatter profile. Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

Analysis of IRAK4 in human epithelioid carcinoma (center left panel). *HeLa S3 cells (ATCC CCL 2.2) were either transfected with IRAK4 RNAi-transfected HeLaS3 cells (open histogram) or untreated (shaded histogram). 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 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-Human IRAK4. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Confirmation of the specificity of mAb L29-525 by western blot analysis (center right panel). *Lysates of human PBMC were probed with unconjugated antibody at a concentration of 0.1 µg/ml. IRAK4 is identified as a band of about 52 kDa.*

Western blot validation of IRAK4 by RNAi (far right panel). *Lysates from HeLa S3 cells (lane 1) and IRAK4 RNAi-transfected HeLa S3 cells (lane 2) were probed with unconjugated mAb L29-525 at 0.015 µg/ml. Down-regulation of IRAK4 expression is evident in the RNAi-transfected cells.*

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

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

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was 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

Recommended Assay Procedure:
This antibody conjugate is suitable for intracellular staining of human peripheral blood mononuclear cells and cell lines (using BD™ Phosflow Lyse/Fix Buffer or BD Cytofix™ Fixation Buffer), but not human whole blood. BD™ Phosflow Perm Buffer II or Perm Buffer III may be used.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558049	Lyse/Fix Buffer 5X	250 ml	(none)
558052	Perm Buffer II	125 ml	(none)
558050	Perm Buffer III	125 ml	(none)
557783	Alexa Fluor® 647 Mouse IgG1 κ Isotype control	50 tests	MOPC-21

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10e6 cells in a 100-µl experimental sample (a test).
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
4. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
5. 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.
6. 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.
7. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Hatao F, Muroi M, Hiki N, Ogawa T, Mimura Y, Kaminishi M, Tanamoto K. Prolonged Toll-like receptor stimulation leads to down-regulation of IRAK-4 protein. *J Leukoc Biol.* 2004; 76(4):904-908.(Biology)
Li S, Strelow A, Fontana EJ, Wesche H. IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. *Proc Natl Acad Sci U S A.* 2002; 99(8):5567-5572.(Biology)
Lye E, Mirtsos C, Suzuki N, Suzuki S, Yeh WC. The role of interleukin 1 receptor-associated kinase-4 (IRAK-4) kinase activity in IRAK-4-mediated signaling. *J Biol Chem.* 2004; 279(39):40653-40658.(Biology)
Suzuki N, Saito T. IRAK-4 – a shared NF-κB activator in innate and acquired immunity. *Trends Immunol.* 2006; 27(12):566-572.(Biology)