

IRF-4 Antibody

✓ 100 µl
(10 Western mini-blot)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 04/21/09

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

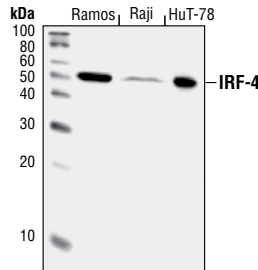
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP, IF-IC, ChIP, F	H	51 kDa	Rabbit**

Background: Interferon regulatory factors (IRFs) are a family of transcription factors that function with the Jak/Stat pathway to regulate interferons in response to viral infection and also regulate interferon-inducible gene expression (1,2). IRFs play an important role in the defense against pathogens, autoimmunity, lymphocyte development, cell growth and susceptibility to transformation. The IRF family has nine members: IRF-1, IRF-2, ISGF3gamma/p48, IRF-3, IRF-4 (Pip/LSIRF/ICSAT), IRF-5, IRF-6, IRF-7 and IRF-8/IC-SBP, which share homology in their amino-terminal DNA binding domains. IRF family members regulate transcription through interactions with proteins which have similar DNA binding motifs including IFN stimulated response element (ISRE), IFN consensus sequence (ICS) and IFN regulatory element (IRF-E).

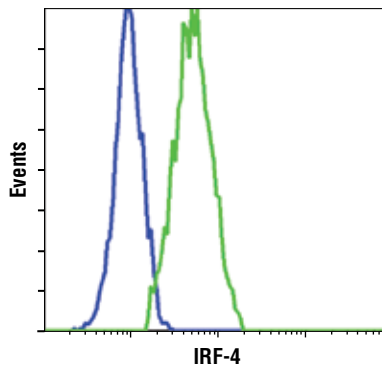
IRF-4 was independently cloned by three groups and demonstrated to have roles in different contexts of lymphoid regulation (3-5). First, IRF-4 (Pip) was found to associate with PU.1, a hematopoietic specific member of the ETS family, and to regulate the expression of B-cell specific genes (3). Second, it was characterized as a lymphoid-specific member of the IRF family (LSIRF) and able to bind to ISRE (4). Third, it was identified in activated T cells as a factor that binds to the promoter of the interleukin-5 gene (ICSAT), and shown to repress gene activation induced by IFN (5). IRF-4 is expressed in all stages of B cell development and in mature T cells, and is inducible in primary lymphocytes by antigen mimetic stimuli such as Concavalin A, CD3 cross-linking, anti-IgM and PMA treatment (4,5). Mice deficient in IRF-4 show normal distribution of B and T lymphocytes at 4 to 5 weeks, but later develop progressive generalized lymphadenopathy, suggesting a role for IRF-4 in the function and homeostasis of mature B- and T-lymphocytes (6).

Specificity/Sensitivity: IRF-4 Antibody detects endogenous levels of IRF-4 protein. The antibody does not cross-react with other family members at physiological levels.

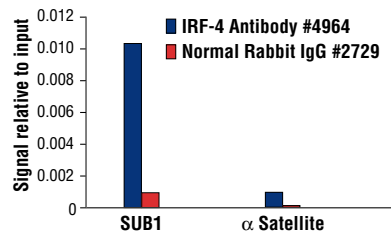
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues around Asp175 of human IRF-4. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from Ramos, Raji, and HuT-78 cells, using IRF-4 Antibody.



Flow cytometric analysis of THP-1 cells (blue) and RPMI 8226 cells (green) using IRF-4 Antibody.



Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western Blotting	1:1000
Immunoprecipitation	1:100
Immunofluorescence (IF-IC)	1:400
Chromatin IP	1:25
Flow Cytometry	1:400

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

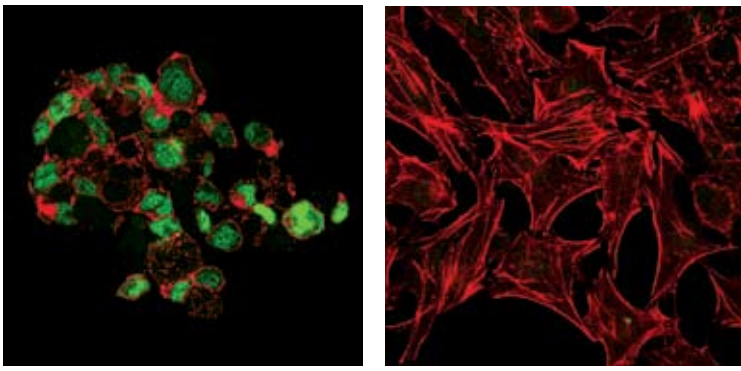
Background References:

- (1) Taniguchi, T. et al. (2001) *Annu. Rev. Immunol.* 19, 623–655.
- (2) Mamane, Y. et al. (1999) *Gene* 237, 1–14.
- (3) Eisenbeis, C.F. et al. (1995) *Genes Dev.* 9, 1377–1387.
- (4) Matsuyama, T. et al. (1995) *Nucleic Acids Res.* 23, 2127–2136.
- (5) Yamagata, T. et al. (1996) *Mol. Cell. Biol.* 16, 1283–1294.
- (6) Mittrucker, H. et al. (1997) *Science* 275, 540–543.

Chromatin immunoprecipitations were performed with cross-linked chromatin from 4×10^6 H929 cells and either 20 µl of IRF-4 Antibody #4964 or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP™ Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by Real-Time PCR using primers specific for the SUB1 gene and the heterochromatic α Satellite repeat element. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA E-P—ELISA Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



Confocal immunofluorescent analysis of RPMI 8226 (left) and HeLa cells (right) using IRF-4 Antibody (green). Actin filaments have been labeled with DY-554 phalloidin (red).