

This product is for *in vitro* research use only and is not intended for use in humans or animals.

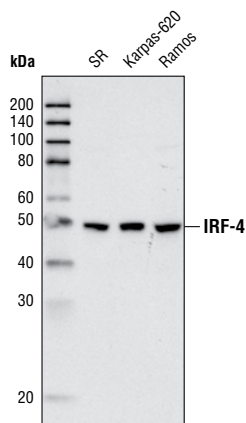
Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H, M, R, (Mk)	50 kDa	Rabbit

Background: Interferon regulatory factors (IRFs) comprise a family of transcription factors that function with the Jak/Stat pathway to regulate interferon (IFN) and IFN-inducible gene expression in response to viral infection (1). IRFs play an important role in the pathogen defense, autoimmunity, lymphocyte development, cell growth and susceptibility to transformation. The IRF family includes nine members: IRF-1, IRF-2, ISGF3γ/p48, IRF-3, IRF-4 (Pip/LSIRF/ICSAT), IRF-5, IRF-6, IRF-7 and IRF-8/ICSBP. All IRF proteins share homology in their amino-terminal DNA binding domains. IRF family members regulate transcription through interactions with proteins that share similar DNA binding motifs, such as IFN stimulated response elements (ISRE), IFN consensus sequences (ICS) and IFN regulatory elements (IRF-E) (2).

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 member of the ETS family specific to hematopoietic cells, 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 Conavalin A, CD3 crosslinking, 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 (P173) Antibody detects endogenous levels of total IRF-4 protein. This 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 Pro173 of human IRF-4. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from SR, Karpas-620 and Ramos cell types using IRF-4 (P173) Antibody.

Background References:

- (1) Taniguchi, T. et al. (2001) *Annu. Rev. Immunol.* 19, 623-655.
- (2) Honda, K. and Taniguchi, T. (2006) *Nat. Rev. Immunol.* 6, 644-658.
- (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) Mittrücker, H.W. et al. (1997) *Science* 275, 540-543.

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.

Recommended Antibody Dilutions:

Western blotting 1:1000

Companion Products:

- IRF-4 Antibody #4964
- Phospho-IRF-3 (Ser396) (4D4G) Rabbit mAb #4947
- IRF-3 Antibody #4962
- IRF-5 Antibody #3257
- Phototope[®]-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO[®] Reagent and 20X Peroxide #7003

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 IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFI[®]

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.