

# Product Datasheet

## IRF5 Antibody NB100-1092

Unit Size: 0.1 mg

Store at -20C. Avoid freeze-thaw cycles.

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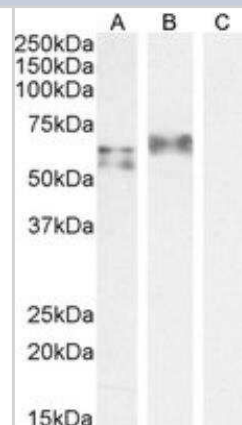


**NB100-1092****IRF5 Antibody**

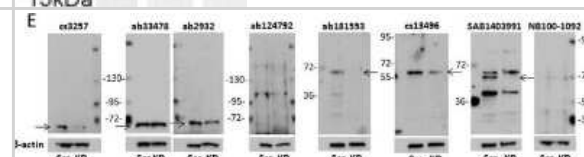
<b>Product Information</b>	
<b>Unit Size</b>	0.1 mg
<b>Concentration</b>	0.5 mg/ml
<b>Storage</b>	Store at -20C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	Tris saline (20 mM Tris pH 7.3, 150 mM NaCl), 0.5% BSA
<b>Target Molecular Weight</b>	56 kDa
<b>Product Description</b>	
<b>Host</b>	Goat
<b>Gene ID</b>	3663
<b>Gene Symbol</b>	IRF5
<b>Species</b>	Human, Mouse
<b>Reactivity Notes</b>	Mouse reactivity reported in scientific literature (PMID: 22879997).
<b>Specificity/Sensitivity</b>	This antibody is expected to recognize all four reported isoforms (NP_002191.1; NP_116032.1; NP_001092097.1; NP_001092099.1). Reported variants NP_001092100.1, NP_001092101.1, NP_001092098.1, NP_001092097.1 represent identical protein: Reported variants NP_116032.1, NP_001092100.1 represent identical protein
<b>Immunogen</b>	Peptide with sequence C-QGPWPMHPAGMQ corresponding to C-Terminus according to NP_002191.1, NP_116032.1, NP_001092097.1, NP_001092099.1.
<b>Product Application Details</b>	
<b>Applications</b>	Western Blot, Flow Cytometry, Peptide ELISA, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
<b>Recommended Dilutions</b>	Western Blot 0.3 - 1 ug/mL, Flow Cytometry, Peptide ELISA Detection limit 1:128000, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
<b>Application Notes</b>	Western blot: Approx 55+60 kDa bands observed in Human spleen lysates and approx 65 kDa in lysates of cell line A549 (calculated MW of 57.8 kDa according to NP_001092099.1 and 56.0 kDa according to NP_116032.1; NP_001092097.2). Use in ChIP reported in scientific literature (PMID: 20237317). Use in Flow cytometry reported in scientific literature (PMID: 22879997).

## Images

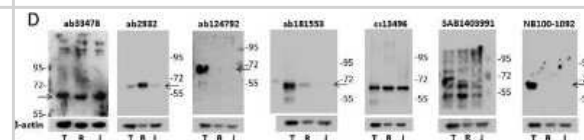
Western Blot: IRF5 Antibody [NB100-1092] - Western blot analysis of Human Spleen (A) and A549 cell lysate (B) + peptide (C), 35 ug protein in RIPA buffer. Antibody at 1 ug/mL. Detected by chemiluminescence



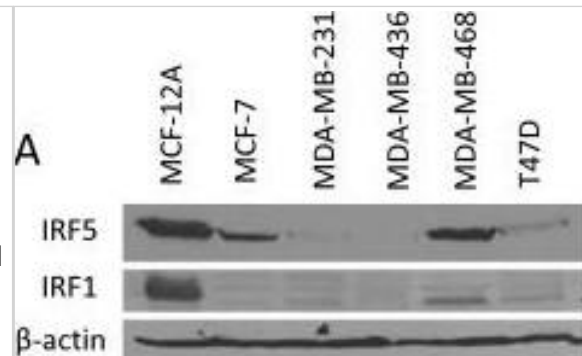
Western Blot: IRF5 Antibody [NB100-1092] - Endogenous IRF5 knockdown analysis. Ramos B cells were nucleofected with scrambled (Scr) or IRF5 (KD) siRNAs and lysates from same nucleofection run on multiple independent blots for comparative analysis of seven different commercially available alpha-IRF5 antibodies. Arrows indicate detection of an appropriately sized band(s) corresponding to IRF5. Data are representative of three independent experiments. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep31002>), licensed under a CC-BY license.



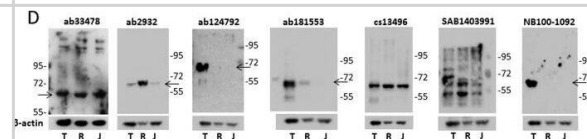
Western Blot: IRF5 Antibody [NB100-1092] - Comparative analysis of IRF5 protein expression between immortalized lymphoid cell lines by immunoblot. Endogenous IRF5 protein expression in lysates (T, THP-1; R, Ramos B cells; J, Jurkat T cells) was detected by running multiple independent blots for the analysis of antibody specificity; seven different commercially available alpha-IRF5 antibodies were evaluated. IRF5 expression was detected with the previously validated Cell Signaling antibody #3257 that is no longer available. beta-actin levels and apparent molecular weight standards are shown as loading controls and for size comparison, respectively. Arrows indicate detection of an appropriately sized band(s) corresponding to IRF5. Data are representative of three independent experiments. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep31002>), licensed under a CC-BY license.



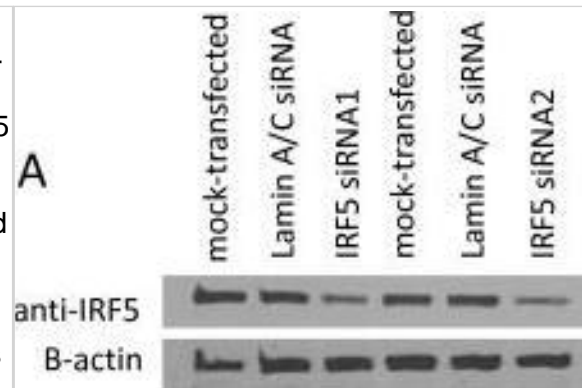
Western Blot: IRF5 Antibody [NB100-1092] - Overexpression of IRF5 in MCF-7 & MDA-MB-231 cells sensitizes them to DNA damage-induced growth inhibition. A. Endogenous IRF expression was analyzed by Western blot in transformed mammary epithelial cell lines. Levels of  $\beta$ -actin are shown as loading controls. B. Western blot analysis of stable cell lines generated to overexpress retroviral pBIRF5. C. Cell survival was measured in MCF-7 & MDA-MB-231 pBabe cell lines by colony formation assay before & after treatment. Cells were treated with 0.1 or 1  $\mu$ M Doxorubicin (Dox) or 2, 5 & 10 Gy  $\gamma$ -IR. The number of colonies is plotted on the y-axis as percent of control; 100% represents the number of colonies in empty pBabe control lines. Data are expressed as mean  $\pm$  SD of three independent experiments performed in duplicate. Statistical significance was determined by comparing the difference between colonies in pBabe versus pBIRF5 cell lines after each treatment; \* denotes  $P < 0.05$ , \*\*  $P < 0.001$ . Image collected & cropped by CiteAb from the following publication (<http://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr3053>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



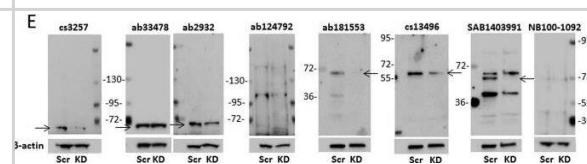
Western Blot: IRF5 Antibody [NB100-1092] - Comparative analysis of IRF5 protein expression between immortalized lymphoid cell lines by immunoblot. (A) qRT-PCR analysis of IRF5 transcript expression in immortalized lymphoid cell lines. Data is presented as relative expression after normalization to  $\beta$ -actin using the  $\Delta\Delta C_t$  method. (B) Immunoblot analysis of endogenous IRF5 protein expression in immortalized cell lines. IRF5 expression was detected with the previously validated Cell Signaling antibody #3257 that is no longer available.  $\beta$ -actin levels & apparent molecular weight standards are shown as loading controls & for size comparison, respectively. (C) Experimental details for the comparative analysis of  $\alpha$ -IRF5 antibody specificity by immunoblot analysis. (D) Same as in (B) except lysates (T, THP-1; R, Ramos B cells; J, Jurkat T cells) were run on multiple independent blots for the analysis of antibody specificity; seven different commercially available  $\alpha$ -IRF5 antibodies were evaluated. (E) Same as in (D) except antibodies were evaluated by IRF5 knockdown analysis. Ramos B cells were nucleofected with scrambled (Scr) or IRF5 (KD) siRNAs & lysates from same nucleofection run on multiple independent blots for comparative analysis. Arrows indicate detection of an appropriately sized band(s) corresponding to IRF5. Data (except in (C)) are representative of three independent experiments. Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep31002>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



**Western Blot: IRF5 Antibody [NB100-1092] - Down-regulation of IRF5 protein expression by siRNAs alters sensitivity to DNA damage.** A. MCF-12A cells were incubated with transfection reagent alone (mock-transfected), control Lamin A/C siRNAs or 5 nM IRF5 siRNAs once (IRF5 siRNA1) or twice (IRF5 siRNA2), as described in the Materials & methods. Western blot analysis shows > 70% reduction of endogenous IRF5 proteins after normalization to  $\beta$ -actin levels. B. Cells were exposed to 5 Gy IR or the same dose plus IFN- $\gamma$  (IR/ $\gamma$ ) for 24 h. Percent of Annexin V-FITC stained positive cells is shown in the upper & lower right-hand quadrants. Representative histogram plots from three independent experiments performed in duplicate are shown. C. Same as in B, except cells were exposed to 1  $\mu$ M Dox or Dox & IFN- $\gamma$  for five hours. Percent of Annexin V-FITC stained positive cells compared to control is plotted on y-axis. Data are expressed as mean  $\pm$  SD of three independent experiments performed in duplicate. Statistical significance was determined by comparing the difference between cells transfected with Lamin A/C siRNAs (12Asicon) & IRF5 siRNAs (12AsiIRF5) after each treatment; \*\* denotes  $P < 0.001$ . D. Cells were treated with the indicated doses of Dox or IR after siRNA transfection. Number of colonies is plotted on y-axis as percent of control. A total of 100% represents the number of colonies in control untreated 12Asicon cells. Data are expressed as mean  $\pm$  SD of three independent experiments performed in duplicate. Statistical significance was determined by comparing the difference between colonies in 12Asicon versus 12AsiIRF5 cells after each treatment; \* denotes  $P < 0.05$ . Image collected & cropped by CiteAb from the following publication (<http://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr3053>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

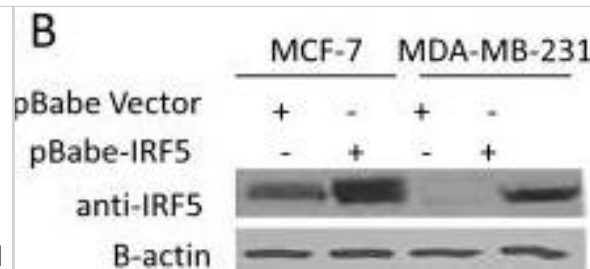


**Western Blot: IRF5 Antibody [NB100-1092] - Comparative analysis of IRF5 protein expression between immortalized lymphoid cell lines by immunoblot.** (A) qRT-PCR analysis of IRF5 transcript expression in immortalized lymphoid cell lines. Data is presented as relative expression after normalization to  $\beta$ -actin using the  $\Delta\Delta C_t$  method. (B) Immunoblot analysis of endogenous IRF5 protein expression in immortalized cell lines. IRF5 expression was detected with the previously validated Cell Signaling antibody #3257 that is no longer available.  $\beta$ -actin levels & apparent molecular weight standards are shown as loading controls & for size comparison, respectively. (C) Experimental details for the comparative analysis of  $\alpha$ -IRF5 antibody specificity by immunoblot analysis. (D) Same as in (B) except lysates (T, THP-1; R, Ramos B cells; J, Jurkat T cells) were run on multiple independent blots for the analysis of antibody specificity; seven different commercially available  $\alpha$ -IRF5 antibodies were evaluated. (E) Same as in (D) except antibodies were evaluated by IRF5 knockdown analysis. Ramos B cells were nucleofected with scrambled (Scr) or IRF5 (KD) siRNAs & lysates from same nucleofection run on multiple independent blots for comparative analysis. Arrows indicate detection of an appropriately sized band(s) corresponding to IRF5. Data (except in (C)) are representative of three independent experiments. Image collected & cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep31002>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.





Western Blot: IRF5 Antibody [NB100-1092] - Overexpression of IRF5 in MCF-7 & MDA-MB-231 cells sensitizes them to DNA damage-induced growth inhibition. A. Endogenous IRF expression was analyzed by Western blot in transformed mammary epithelial cell lines. Levels of  $\beta$ -actin are shown as loading controls. B. Western blot analysis of stable cell lines generated to overexpress retroviral pBIRF5. C. Cell survival was measured in MCF-7 & MDA-MB-231 pBabe cell lines by colony formation assay before & after treatment. Cells were treated with 0.1 or 1  $\mu$ M Doxorubicin (Dox) or 2, 5 & 10 Gy  $\gamma$ -IR. The number of colonies is plotted on the y-axis as percent of control; 100% represents the number of colonies in empty pBabe control lines. Data are expressed as mean  $\pm$  SD of three independent experiments performed in duplicate. Statistical significance was determined by comparing the difference between colonies in pBabe versus pBIRF5 cell lines after each treatment; \* denotes  $P < 0.05$ , \*\*  $P < 0.001$ . Image collected & cropped by CiteAb from the following publication (<http://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr3053>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Bi Xiaohui, Hameed Meera, Mirani Neena et al. Loss of interferon regulatory factor 5 (IRF5) expression in human ductal carcinoma correlates with disease stage and contributes to metastasis. *Breast Cancer Res* 2011-01-01 [PMID: 22053985] (WB, Human)

Li D, De S, Li D et al. Specific detection of interferon regulatory factor 5 (IRF5): A case of antibody inequality. *Sci Rep* 2016-08-02 [PMID: 27481535]

Barnes BJ, Moore PA, Pitha PM. Virus-specific activation of a novel interferon regulatory factor, IRF-5, results in the induction of distinct interferon alpha genes. *J Biol Chem* 2001-06-29 [PMID: 11303025]

Hanke ML, Angle A, Kielian T. MyD88-Dependent Signaling Influences Fibrosis and Alternative Macrophage Activation during *Staphylococcus aureus* Biofilm Infection *plos One* 2012-01-01 [PMID: 22879997] (FLOW, Mouse)

Ye L, Peng JS, Wang X, Wang YJ, Luo GX, Ho WZ. Methamphetamine enhances Hepatitis C virus replication in human hepatocytes. *J Viral Hepat*;15(4):261-70. 2008-04-01 [PMID: 18307590]

Ning S, Huye LE, Pagano JS. Interferon regulatory factor 5 represses expression of the Epstein-Barr virus oncoprotein LMP1: braking of the IRF7/LMP1 regulatory circuit. *J Virol*;79(18):11671-6. 2005-09-01 [PMID: 16140744]

Krausgruber T, Saliba D, Ryzhakov G, Lanfrancotti A, Blazek K, Udalova IA. IRF5 is required for late-phase TNF secretion by human dendritic cells. *Blood*;115(22):4421-30. 2010-06-03 [PMID: 20237317] (Chemotaxis)

Hu G, Barnes BJ. IRF-5 is a mediator of the death receptor-induced apoptotic signaling pathway. *J Biol Chem*;284(5):2767-77. 2009-01-30 [PMID: 19028697]

Graham RR et al. Three functional variants of IFN regulatory factor 5 (IRF5) define risk and protective haplotypes for human lupus. *Proc Natl Acad Sci U S A*;104(16):6758-63. 2007-04-17 [PMID: 17412832]



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### **Products Related to NB100-1092**

NBL1-12034	IRF5 Overexpression Lysate
HAF017	Rabbit anti-Goat IgG Secondary Antibody [HRP (Horseradish Peroxidase)]
HAF109	Donkey anti-Goat IgG Secondary Antibody [HRP (Horseradish Peroxidase)]
NB410-28088-1mg	Goat IgG Isotype Control

### **Limitations**

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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