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MYD88 Antibody

Cat. No.: 2127

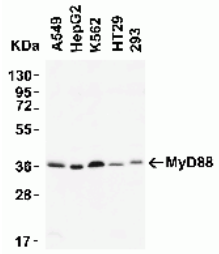

Figure 1 Western Blot Validation of MyD88 in human cell lines
Loading: 15 ug of lysates per lane. Antibodies: 2127 (2 ug/mL) 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution. Predicted band size: 35 kDa

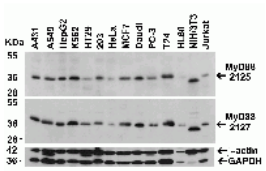

Figure 2 Independent Antibody Validation (IAV) via Protein Expression Profile in Cell Lines
Loading: 15 ug of lysates per lane. Antibodies: MyD88 2125 (2 ug/mL), MyD88 2127 (2 ug/mL), beta-actin (1 ug/mL), and GAPDH (0.02 ug/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

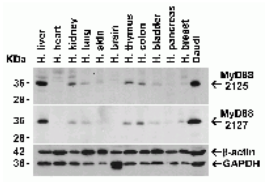

Figure 3 Independent Antibody Validation (IAV) via Protein Expression Profile in Human Tissues
Loading: 15 ug of lysates per lane. Antibodies: MyD88 2125 (2 ug/mL), MyD88 2127 (2 ug/mL), beta-actin (1 ug/mL), and GAPDH (0.02 ug/mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

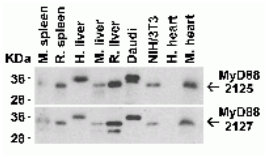

Figure 4 Animal Species Reactivity
Loading: Lysates/proteins at 15 ug per lane. Antibodies: 2125 (2 ug/mL) or 2127 (2 ug/mL). 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

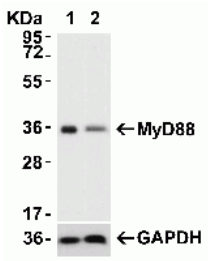

Figure 5 Validation with MyD88 siRNA Knockdown
HeLa cells were transfected with control siRNAs (lane 1) or MyD88 siRNAs (lane 2) Loading: 10 ug of HeLa whole cell lysates per lane. Antibodies: 2127 (2 ug /mL), 1 h incubation at RT in 5% NFDM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10000 dilution.

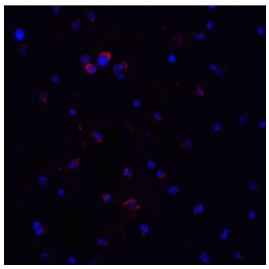

Figure 6 Immunofluorescence Validation of MyD88
Immunofluorescent analysis of 4% paraformaldehyde-fixed Jurkat cells labeling MyD88 with 2127 at 20 ug/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red) and DAPI staining (blue).

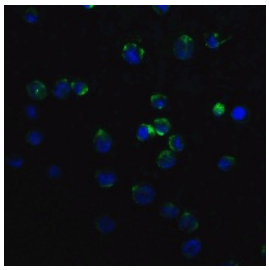

Figure 6 Immunofluorescence Validation of MyD88 in Jurkat Cells
Immunofluorescent analysis of 4% paraformaldehyde-fixed Jurkat cells labeling MyD88 with 2127 at 20 ug/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red) and DAPI staining (blue).

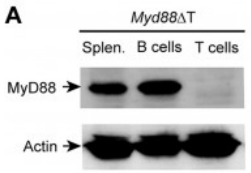

Figure 8 KO Validation of MyD88 in T cells (Rahman et al., 2011)
Splenocytes were isolated from a Myd88ΔT mouse in which MyD88 was specifically disrupted in T cells. T and B cells were FACS purified, and MyD88 expression was examined by Western blot with anti-MyD88 antibodies (2127). MyD88 expression was detected in Splenocytes and B cells, but not in T cells.

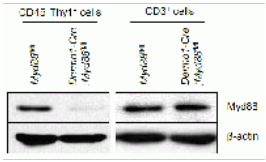

Figure 9 KO Validation in CD45-Thy1+ cells (Kim et al., 2017)
The expression of Myd88 protein was analyzed in CD45−Thy1+ intestinal mesenchymal cells and CD3+ intestinal T cells, by immunoblotting with anti-Myd88 antibodies (2127). MyD88 expression was not detected in knockout cells.

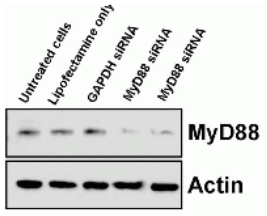

Figure 10 KD Validation in Raw 264.7 cells (Altimeier et al., 2007)
The Transfection of RAW 264.7 cells with MyD88-specific siRNA resulted in attenuation of MyD88 protein by Western blot analysis with anti-Myd88 antibodies (2127).

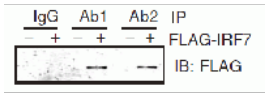

Figure 11 Immunoprecipitation Validation in HEK293 cells (Kawai et al., 2004)
HEK293 cells were transiently transfected with FLAG-IRF7. Ccell lysates were immunoprecipitated with control rabbit anti-mouse immunoglobulin serum (IgG) or anti-MyD88 (Ab1 and Ab2), followed by immunoblotting with anti-FLAG.

ΨSPECIFICATIONS

HOST SPECIES:	Rabbit
SPECIES REACTIVITY:	Human, Mouse, Rat
HOMOLOGY:	Predicted species reactivity based on immunogen sequence: Pig: (94%), Sheep: (82%), Bovine: (82%), Chicken: (82%)
IMMUNOGEN:	Anti-MYD88 antibody (2127) was raised against a peptide corresponding to 17 amino acids near carboxy terminus of human MYD88 isoform 1. The immunogen is located within the last 50 amino acids of MYD88.
TESTED APPLICATIONS:	ELISA, IF, IP, WB
APPLICATIONS:	WB: 0.5 - 2 ug/mL; IF: 20 ug/mL. Antibody validated: Western Blot in human, mouse and rat samples; Immunofluorescence and Immunoprecipitation in human samples. All other applications and species not yet tested.
POSITIVE CONTROL:	1) Cat. No. 1202 - A431 Cell Lysate
	2) Cat. No. 1203 - A549 Cell Lysate
	3) Cat. No. 1204 - K562 Cell Lysate
	4) Cat. No. 1211 - HepG2 Cell Lysate
	5) Cat. No. 1282 - NIH/3T3 Cell Lysate
PREDICTED MOLECULAR WEIGHT:	Predicted: 35kD Observed: 35kD

ΨADVANCED VALIDATION

VALIDATION:	**Independent Antibody Validation in Cell lines**(Figure 2) shows similar MYD88 expression profile in both human and mouse cell lines detected by two independent anti-MYD88 antibodies that recognize different epitopes, **2125** against internal domain and **2127** against the C-terminus domain. MYD88 proteins are detected in all the tested cell lines at different expression levels by the two independent antibodies. Additionally, Figure 2 shows the mouse MYD88 protein in NIH/3T3 cells migrates slightly faster than human isoform 1 detected by both MYD88 antibodies (2125 and 2127), which is well correlated with their calculated molecular masses (33.8 kDa vs 35.4 kDa). **Independent Antibody Validation in Human Tissues**(Figure 3) shows similar MYD88 expression profile in human tissues detected by two independent anti-MYD88 antibodies (2125 and 2127). MYD88 proteins are detected by the two independent antibodies in liver, kidney, lung, thymus, colon, bladder and breast of human tissues at different expression levels, but not in heart, brain, skin and pancreas. **Animal Species Reactivity** (Figure 4): Anti-MYD88 antibodies (**2125** and **2127**) can detect the expression of MYD88 protein in the liver and spleen of all tissues and mouse heart, but not in human heart. Additionally, Figure 4 also shows MYD88 protein detected by both MYD88 antibodies (2125 and 2127) in human liver and Daudi cells migrates slightly slower than that in the tissues of mouse and rat, which is well correlated with their calculated molecular masses (35.4 kDa vs 33.8 kDa and 33.9kD). **siRNA knockdown validation** (Figure 5): Anti-MYD88 antibody (**2127**) specificity was further verified by MYD88 specific siRNA knockdown. MYD88 signal in HeLa cells transfected with MYD88 siRNAs was weaker in comparison with that in HeLa cells transfected with control siRNAs.
ISOFORMS:	Human MYD88 has 7 isoforms, including isoform 1 (317aa, 35.4kD), isoform 2 (296aa, 33.2 kD), isoform 3 (251aa, 28.3kD), isoform 4 (191aa, 20.8kD), isoform 5 (146aa, 15.8kD), isoform 6 (275aa, 31.5kD), and isoform 7 (304aa, 34.1kD). This antibody detects human isoform 1,2,3,6,7, but not isoform 4,5. Mouse MYD88 has two isoforms, including isoform 1 (296aa, 33.8kD) and isoform 2 (250aa, 28.7kD). Rat MYD88 has only one isoform identified so far (296aa, 33.9kD).

ΨPROPERTIES

PURIFICATION:	MYD88 Antibody is affinity chromatography purified via peptide column.
CLONALITY:	Polyclonal
ISOTYPE:	IgG
CONJUGATE:	Unconjugated
PHYSICAL STATE:	Liquid
BUFFER:	MYD88 Antibody is supplied in PBS containing 0.02% sodium azide.
CONCENTRATION:	1 mg/mL
STORAGE CONDITIONS:	MYD88 antibody can be stored at 4˚C for three months and -20˚C, stable for up to one year. As with all antibodies care should be taken to avoid repeated freeze thaw cycles. Antibodies should not be exposed to prolonged high temperatures.

ΨADDITIONAL INFO

OFFICIAL SYMBOL:	MYD88
ALTERNATE NAMES:	MYD88 Antibody: MYD88D
ACCESSION NO.:	AAB49967.1
PROTEIN GI NO.:	1763090
GENE ID:	4615
USER NOTE:	Optimal dilutions for each application to be determined by the researcher.

ΨBACKGROUND AND REFERENCES

BACKGROUND:	MYD88, myeloid differentiation primary response 88, was identified as an innate immune signal transduction adaptor involved in the Toll-like receptor (TLR) and interleukin-1 (IL-1) signaling pathway (1,2,3) and plays an important role in the inflammatory response induced by cytokines IL-1 and IL-18 and endotoxin. MyD88 functions as an adaptor protein for TLRs and IL-1 receptors, which stimulates IRAKs, IRF7 and TRAF6, leading to NF-κB activation, cytokine secretion and inflammatory response (2, 4,5,6). Nuclear factor-kappa-B activation modulates multiple genes regulating the body's immune reactions and inflammatory responses. MyD88 associates with and recruits IRAK to the IL-1 receptor complex in response to IL-1 treatment and dominant negative form of MyD88 attenuates IL-1R-mediated NF-κB activation(4,5). MyD88 is also employed as a regulator molecule by IL-18 receptor. Targeted disruption of the MyD88 gene results in lose of cellular responses to IL-1 and IL-18, and MyD88-deficient mice lack responses to bacterial product LPS that employs TLR2 and TLR4 as the signaling receptors(7,8). MyD88 gene is expressed in many tissues.
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ΨCITATIONS

CITATIONS:	1)Rahman et al. Antiviral memory CD8 T-cell differentiation, maintenance, and secondary expansion occur independently of MyD88. Blood. 2011; 117(11): 3123-30. PMID: 21233312
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