# **Product Datasheet**

# IL-17E/IL-25 Antibody (68C1039.2) - BSA Free NB100-56541

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Updated 10/23/2024 v.20.1

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## NB100-56541

IL-17E/IL-25 Antibody (68C1039.2) - BSA Free

Product Information					
Unit Size	0.1 mg				
Concentration	1.0 mg/ml				
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.				
Clonality	Monoclonal				
Clone	68C1039.2				
Preservative	0.05% Sodium Azide				
Isotype	lgG1				
Purity	Protein G purified				
Buffer	PBS				
Target Molecular Weight	20 kDa				
Product Description					
Host	Mouse				
Gene ID	64806				
Gene Symbol	IL25				
Species	Human, Mouse				
Immunogen	Amino acids 115-132 of human IL-17E (isoform CRA_a) were used as immunogen for this antibody.				
Product Application Details					
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin				
Recommended Dilutions	Dilutions Western Blot 1:100 - 1:250, Immunohistochemistry 1:100 - 1:500, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 35359953), Immunohistochemistry-Paraffin reported in scientific literature (Terrier B et al)				
Application Notes	The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.				
Imagaa					

#### Images

Western Blot: IL-17E/IL-25 Antibody (68C1039.2) [NB100-56541] - Analysis of IL-17E using IL-17E monoclonal antibody. Mouse testis lysate probed with IL-17E antibody at 2 ug/mL.					

MW (kDa) 200 —			
116 97 =			
66 -			
55 -			
36 -			
31 -			
21			
14			
6-			







Α Western Blot: IL-17E/IL-25 Antibody (68C1039.2) - BSA Free [NB100-56541] - IL25 upregulated GLI1 by inhibiting p-AMPK. (A) HT-29 cells CON IL25(50ng/ml) were treated with cycloheximide (CHX, 50 µg/ml) for the indicated time, 0 15 30 60 90 0 15 30 60 90 (min) CHX & cell lysates were analyzed by Western blotting with the indicated GLI1 117KD antibodies. (B) Western blotting of p-AMPK & AMPK in the WT & IL25KO AOM/DSS-induced tumor tissue. (C) The expression levels of GLI1, p-GAPDH 35KD AMPK, & AMPK were examined in HT-29 & SW620 cells treated with T<sub>1/2</sub>>90min T<sub>1/2</sub>=23min recombinant IL25 in a time-dependent manner by Western blotting. (D, HT-29 E) Western blotting of GLI1, p-AMPK, & AMPK in SW620 cells treated with AMPK activator A769662 & Metformin following IL25 treatment. (F) GLI1 expression was detected by immunofluorescence staining in SW620 cells treated with AMPK activator Metformin following IL25 treatment. (G) SW620 cells were treated with 10 µM MG132 & then incubated with or without 50 ng/ml recombinant IL25 & 1 mM Metformin, then immunoprecipitated with GLI1 antibody. GLI1 ubiquitination was determined using an anti-ubiquitin antibody. IP, immunoprecipitation. (H) Sphere formation analysis of SW620 cells treated with AMPK activator Metformin following IL25 treatment. Representative images (left) & the mean numbers & sphere size (right) of spheres are shown. Data present as mean ± SEM; \*p <0.05, \*\*p <0.01, \*\*\*p <0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35359953), licensed under a CC-BY license. Not internally tested by Novus Biologicals. IL25(50ng/ml) Western Blot: IL-17E/IL-25 Antibody (68C1039.2) - BSA Free [NB100-IL25(50ng/ml) Time/h 0 4 8 12 24 0 4 8 12 24 56541] - IL25 upregulated GLI1 by inhibiting p-AMPK. (A) HT-29 cells GLI1 117KD were treated with cycloheximide (CHX, 50 µg/ml) for the indicated time, & cell lysates were analyzed by Western blotting with the indicated 62KD p-AMPK antibodies. (B) Western blotting of p-AMPK & AMPK in the WT & IL25KO AOM/DSS-induced tumor tissue. (C) The expression levels of GLI1, p-AMPK 62KD AMPK, & AMPK were examined in HT-29 & SW620 cells treated with GAPDH 35KD recombinant IL25 in a time-dependent manner by Western blotting. (D. HT-29 SW620 E) Western blotting of GLI1, p-AMPK, & AMPK in SW620 cells treated with AMPK activator A769662 & Metformin following IL25 treatment. (F) GLI1 expression was detected by immunofluorescence staining in SW620 cells treated with AMPK activator Metformin following IL25 treatment. (G) SW620 cells were treated with 10 µM MG132 & then incubated with or without 50 ng/ml recombinant IL25 & 1 mM Metformin, then immunoprecipitated with GLI1 antibody. GLI1 ubiquitination was determined using an anti-ubiquitin antibody. IP, immunoprecipitation. (H) Sphere formation analysis of SW620 cells treated with AMPK activator Metformin following IL25 treatment. Representative images (left) & the mean numbers & sphere size (right) of spheres are shown. Data present as mean ± SEM; \*p <0.05, \*\*p <0.01, \*\*\*p <0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35359953), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: IL-17E/IL-25 Antibody (68C1039.2) - BSA Free [NB100-56541] - IL25 upregulated GLI1 by inhibiting p-AMPK. (A) HT-29 cells were treated with cycloheximide (CHX, 50 µg/ml) for the indicated time, & cell lysates were analyzed by Western blotting with the indicated antibodies. (B) Western blotting of p-AMPK & AMPK in the WT & IL25KO AOM/DSS-induced tumor tissue. (C) The expression levels of GLI1, p-AMPK, & AMPK were examined in HT-29 & SW620 cells treated with recombinant IL25 in a time-dependent manner by Western blotting. (D, E) Western blotting of GLI1, p-AMPK, & AMPK in SW620 cells treated with AMPK activator A769662 & Metformin following IL25 treatment. (F) GLI1 expression was detected by immunofluorescence staining in SW620 cells treated with AMPK activator Metformin following IL25 treatment. (G) SW620 cells were treated with 10 µM MG132 & then incubated with or without 50 ng/ml recombinant IL25 & 1 mM Metformin, then immunoprecipitated with GLI1 antibody. GLI1 ubiquitination was determined using an anti-ubiquitin antibody. IP, immunoprecipitation. (H) Sphere formation analysis of SW620 cells treated with AMPK activator Metformin following IL25 treatment. Representative images (left) & the mean numbers & sphere size (right) of spheres are shown. Data present as mean ± SEM; \*p <0.05, \*\*p <0.01, \*\*\*p <0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35359953), licensed under a CC-BY license. Not internally tested by Novus Biologicals. Immunohistochemistry: IL-17E/IL-25 Antibody (68C1039.2) - BSA Free [NB100-56541] - Overexpression of IL25 was found in CRC patients & predicts a poor prognosis. (A) Immunohistochemistry (IHC) staining of IL25 was performed in a tissue microarray consisting of 74 CRC tumor tissues & adjacent colon tissues (left). Statistical analysis of IL25 staining 200> in adjacent specimens & CRC specimens (right). (B) Protein levels of IL25 were detected by Western blotting in normal intestinal cells (CCD841) & CRC cell lines (left). The right panel showed the quantitative analysis of the gray scan. The ImageJ software was used for gray scanning. (C) Representative images of IL25 IHC staining at different clinical stages (up). Correlation between IL25 expression & various clinical stages (down). (D) Overall survival curves of 49 CRC patients in correlation with intra-tumor IL25 IHC-scores. High IL25 expression was considered IHC-Score >6. The patients with CRC were divided into 2 groups according to the intra-tumor IL25 IHC-score: low group (n = 34), high group (n = 15). (E) Representative images of IL25 IHC staining from WT colon & AOM/DSS induced tumors on weeks 10 & 16 (down). Statistical analysis of IL25 staining in con colon, adjacent tissues, & AOM/DSS-induced CRC tissues (up). Data present as mean ± SEM; \*p

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< 0.05, \*\*p < 0.01, \*\*\*p < 0.001. Image collected & cropped by CiteAb





Page 5 of 10 v.20.1 Updated 10/23/2024 IL25(50ng/ml) Western Blot: IL-17E/IL-25 Antibody (68C1039.2) - BSA Free [NB100-56541] - IL25 upregulated GLI1 by inhibiting p-AMPK. (A) HT-29 cells Metformin(1mM) were treated with cycloheximide (CHX, 50 µg/ml) for the indicated time, & cell lysates were analyzed by Western blotting with the indicated 117KD GLI1 antibodies. (B) Western blotting of p-AMPK & AMPK in the WT & IL25KO AOM/DSS-induced tumor tissue. (C) The expression levels of GLI1, p-62KD AMPK, & AMPK were examined in HT-29 & SW620 cells treated with p-AMPK recombinant IL25 in a time-dependent manner by Western blotting. (D, E) Western blotting of GLI1, p-AMPK, & AMPK in SW620 cells treated 62KD AMPK with AMPK activator A769662 & Metformin following IL25 treatment. (F) GLI1 expression was detected by immunofluorescence staining in GAPDH 35KD SW620 cells treated with AMPK activator Metformin following IL25 treatment. (G) SW620 cells were treated with 10 µM MG132 & then SW620 incubated with or without 50 ng/ml recombinant IL25 & 1 mM Metformin, then immunoprecipitated with GLI1 antibody. GLI1 ubiquitination was determined using an anti-ubiquitin antibody. IP, immunoprecipitation. (H) Sphere formation analysis of SW620 cells treated with AMPK activator Metformin following IL25 treatment. Representative images (left) & the mean numbers & sphere size (right) of spheres are shown. Data present as mean ± SEM; \*p <0.05, \*\*p <0.01, \*\*\*p <0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35359953), licensed under a CC-BY license. Not internally tested by Novus Biologicals. Immunohistochemistry: IL-17E/IL-25 Antibody (68C1039.2) - BSA Free П Ш IV [NB100-56541] - Overexpression of IL25 was found in CRC patients & predicts a poor prognosis. (A) Immunohistochemistry (IHC) staining of 50> IL25 was performed in a tissue microarray consisting of 74 CRC tumor tissues & adjacent colon tissues (left). Statistical analysis of IL25 staining in adjacent specimens & CRC specimens (right). (B) Protein levels of IL25 were detected by Western blotting in normal intestinal cells (CCD841) & CRC cell lines (left). The right panel showed the quantitative analysis of the gray scan. The ImageJ software was used for gray scanning. (C) Representative images of IL25 IHC staining at different clinical stages (up). Correlation between IL25 expression & various clinical stages (down). (D) Overall survival curves of 49 CRC patients in correlation with intra-tumor IL25 IHC-scores. High IL25 expression was considered IHC-Score >6. The patients with CRC were divided into 2 groups according to the intra-tumor IL25 IHC-score: low group (n = 34), high group (n = 15). (E) Representative images of IL25 IHC staining from WT colon & AOM/DSS induced tumors on weeks 10 & 16 (down). Statistical analysis of IL25 staining in con colon, adjacent tissues, & AOM/DSS-induced CRC tissues (up). Data present as mean ± SEM; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35359953), licensed under a CC-BY license. Not internally tested by Novus Biologicals.







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#### **Publications**

Liu J, Qian B, Zhou L et al. IL25 Enhanced Colitis-Associated Tumorigenesis in Mice by Upregulating Transcription Factor GLI1 Frontiers in Immunology 2022-03-14 [PMID: 35359953] (ICC/IF, IF/IHC, Human, Mouse)

Yi H Intestinal tuft cells in IBS patients: Relationship to mucosal IgE-expressing mast cells Thesis 1905-07-13 (IF/IHC)

Yi H Intestinal tuft cells in IBS patients: Relationship to mucosal IgE-expressing mast cells Thesis Jul 13 1905 12:00AM (IHC)

Kubo F, Ariestanti DM, Oki S et al. Loss of the adhesion G-protein coupled receptor ADGRF5 in mice induces airway inflammation and the expression of CCL2 in lung endothelial cells Respir. Res. 2019-01-17 [PMID: 30654796] (WB, Mouse)

Liu Y, Sun X, Zhao X et al. Expression and location of IL-17A, E, F and their receptors in colorectal adenocarcinoma: Comparison with benign intestinal disease Pathol Res Pract 2018-04-19 [PMID: 29548809] (Human)

Details:

Citation using the Biotin form of this antibody.

Liu Y, Zhao X, Sun X et al. Expression of IL-17A, E, and F and their receptors in human prostatic cancer: Comparison with benign prostatic hyperplasia. Prostate. 2015-12-01 [PMID: 26356122] (Human)

Details:

This citation used the Biotin version of this antibody.

Terrier B, Bieche I, Maisonobe T et al. Interleukin-25: a cytokine linking eosinophils and adaptive immunity in Churg-Strauss syndrome. Blood. 2010-11-01 [PMID: 20729468] (IHC-P)

Details:

IL-17E (IMG-323A). IHC (paraffin): Nerve tissue from patients with Churg-Strauss syndrome (CSS) or miscroscopic polyangiitis (MPA), Fig 5.



#### **Procedures**

#### Immunohistochemistry-Paraffin Protocol for IL-17E/IL-25 Antibody (NB100-56541) Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:

- 1. Wash sections in deionized water three times for 5 minutes each.
- 2. Wash sections in PBS for 5 minutes.
- 3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
- 4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
- 5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
- 6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
- 7. Wash sections three times in wash buffer for 5 minutes each.
- 8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
- 9. As soon as the sections develop, immerse slides in deionized water.
- 10. Counterstain sections in hematoxylin.
- 11. Wash sections in deionized water two times for 5 minutes each.
- 12. Dehydrate sections.
- 13. Mount coverslips.





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## Products Related to NB100-56541

NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NBL1-11946	IL-17E/IL-25 Overexpression Lysate

#### 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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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