Product Datasheet

Cyr61/CCN1 Antibody - BSA Free
NB100-356

Unit Size: 0.1 ml

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

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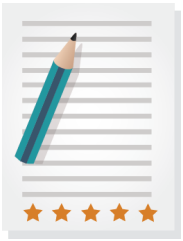
Updated 4/13/2025 v.20.1



NB100-356

Cyr61/CCN1 Antibody - BSA Free

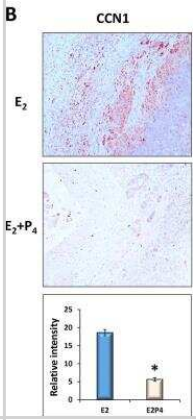
Product Information	
Unit Size	0.1 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	37 kDa

Product Description	
Host	Rabbit
Gene ID	3491
Gene Symbol	CCN1
Species	Human, Mouse, Rabbit
Reactivity Notes	Rabbit reactivity reported in scientific literature (PMID: 22401280). Mouse reactivity reported in scientific literature (PMID: 27776183).
Immunogen	A synthetic peptide made to the human CYR61 protein sequence (between residues 150-250). [UniProt# O00622]

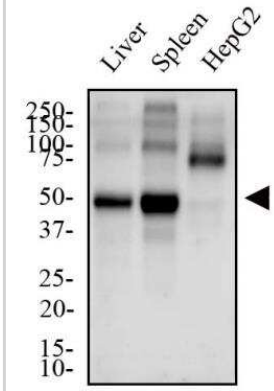
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Block/Neutralize
Recommended Dilutions	Western Blot 1:200, Simple Western 1:400, Immunohistochemistry reported in scientific literature, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 18202125), Immunohistochemistry-Paraffin 1:100, Block/Neutralize reported in scientific literature (PMID 22282654)
Application Notes	In Western blot a band is observed at ~37 kDa, representing the processed form of CYR61 protein. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in MCF-7 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:400, apparent MW was 39 kDa

Images

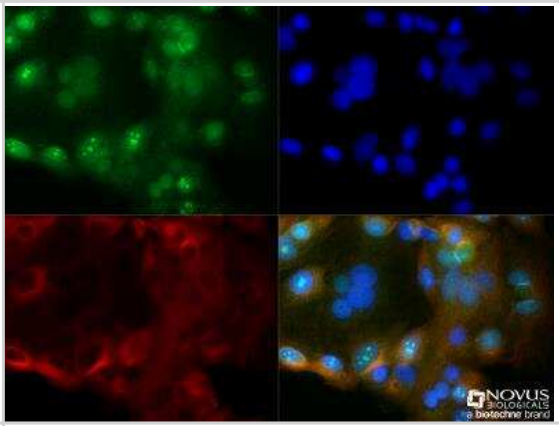
Immunohistochemistry: Cyr61/CCN1 Antibody [NB100-356] - Sections of the ectopic lesions collected from E2 or E2 plus P4-treated recipients (D16, n = 6) were subjected to histological examination. (B) Representative images (20X) showing IHC analysis using antibodies against an angiogenetic regulator CCN1. The immunostaining intensity of CCN1 was analyzed by ImageJ software. The numerical values were analyzed by One-way ANOVA followed by Dunnett's post hoc test and expressed as mean +/- SEM. Statistical significance is defined as *: p<0.01. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0165347) licensed under a CC-BY license.



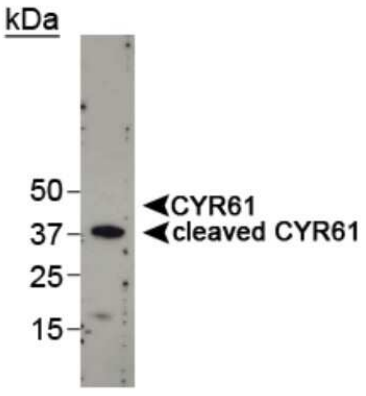
Western Blot: Cyr61/CCN1 Antibody [NB100-356] - Total protein from Human liver, spleen and HepG2 cells was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/mL anti-CYR61 in 1% non-fat milk in TBST and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Note the low expression level of CYR61 in HepG2 cells.

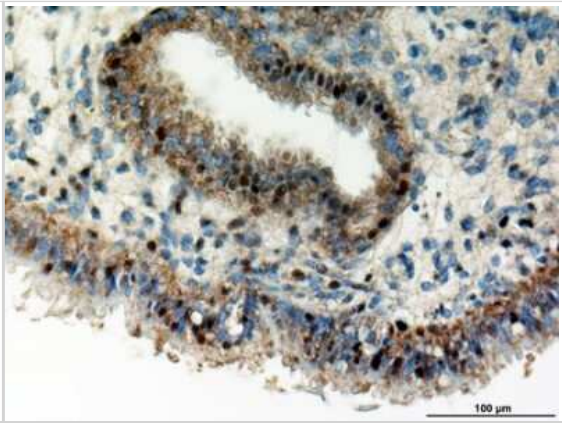
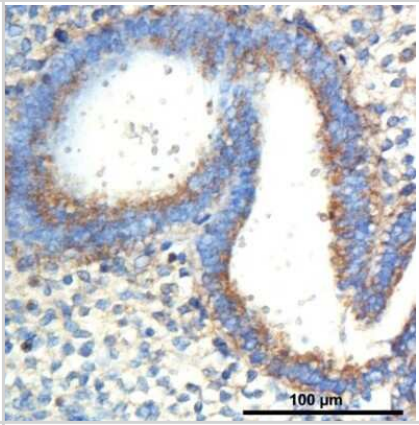
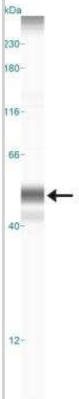
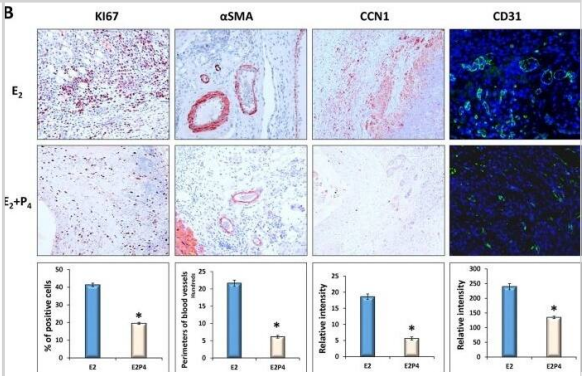


Immunocytochemistry/Immunofluorescence: Cyr61/CCN1 Antibody [NB100-356] - MCF7 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-Cyr61/CCN1 (NB100-356) at 1:200 overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at 1:500. Alpha tubulin was used as a co-stain at 1:1000 and detected with an anti-mouse DyLight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Western Blot: Cyr61/CCN1 Antibody [NB100-356] - Detection of cleaved CYR61 in MDA-MB-231 cell lysate using NB 100-356. Photo courtesy of Dr. Lupu's Lab, Northwestern University. Image by Dr. Ingrid Espinoza.



Immunohistochemistry: Cyr61/CCN1 Antibody [NB100-356] - Human endometrium. Photo courtesy of Dr. rer. nat. Isabella Gashaw, University Duisburg-Essen.	
Immunohistochemistry: Cyr61/CCN1 Antibody [NB100-356] - Human endometrium. Photo courtesy of Dr. rer. nat. Isabella Gashaw, University Duisburg-Essen.	
Simple Western: Cyr61/CCN1 Antibody [NB100-356] - Image shows a specific band for CYR61 in 0.5 mg/mL of MCF-7 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Immunocytochemistry/ Immunofluorescence: Cyr61/CCN1 Antibody - BSA Free [NB100-356] - P4 inhibits E2-dependent cell proliferation & angiogenesis in ectopic lesions.Sections of the ectopic lesions collected from E2 or E2 plus P4-treated recipients (D16, n = 6) were subjected to histological examination. (A) Representative images (20X) showing H&E & Trichrome staining, or IHC analysis using antibody against myofibroblast biomarker αSMA, uterine epithelial biomarker KRT11, or uterine stromal biomarker VIM, respectively. (B) Representative images (20X) showing IHC analysis using antibodies against cell proliferation biomarker KI67, smooth muscle biomarker αSMA, endothelial cells CD31, or an angiogenetic regulator CCN1, respectively. The numbers of KI67-positive cells, the perimeters of the supporting blood vessels, & the immunostaining intensities of CCN1 & CD31 were analyzed by ImageJ software. The numerical values were analyzed by One-way ANOVA followed by Dunnett's post hoc test & expressed as mean ± SEM. Statistical significance is defined as #: $p < 0.05$, *: $p<0.01$. Image collected & cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0165347), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Publications

Heo YJ, Park J, Lee N et Al. Cysteine-rich 61 inhibition attenuates hepatic insulin resistance and improves lipid metabolism in high-fat diet fed mice and HepG2 cells FASEB J 2024-07-31 [PMID: 39082187]

Wang Z, Kim SY, Tu W et al. Extracellular vesicles in fatty liver promote a metastatic tumor microenvironment Cell metabolism 2023-05-09 [PMID: 37172577]

Holmes B, Benavides-Serrato A, Saunders Jt Et Al. mTORC2-mediated direct phosphorylation regulates YAP activity promoting glioblastoma growth and invasive characteristics Neoplasia (New York, N.Y.) 2021-07-31 [PMID: 34343821]

Uneda A, Kurozumi K, Fujimura A et al. Differentiated glioblastoma cells accelerate tumor progression by shaping the tumor microenvironment via CCN1-mediated macrophage infiltration Acta neuropathologica communications 2021-02-22 [PMID: 33618763] (IF/IHC, Human)

Choi C, Jeong W, Ghang B et al. Cyr61 synthesis is induced by interleukin-6 and promotes migration and invasion of fibroblast-like synoviocytes in rheumatoid arthritis Arthritis Res Ther 2020-11-23 [PMID: 33228785] (Human)

Details:
Human fibroblast-like synoviocytes underwent wound-healing and invasiveness assays.

Mugahid D, Kalocsay M, Liu X et al. YAP regulates cell size and growth dynamics via non-cell autonomous mediators Elife 2020-01-09 [PMID: 31913124] (Human)

Hellinger JW, HUchel S, Goetz L et al. Inhibition of CYR61-S100A4 Axis Limits Breast Cancer Invasion Front Oncol. 2019-10-23 [PMID: 31709177] (Human)

Details:
Citation used the Alexa Fluor 488 format of this antibody.

Park MH, Kim AK, Manandhar S et al. CCN1 interlinks integrin and Hippo pathway to autoregulate tip cell activity Elife 2019-08-20 [PMID: 31429823] (B/N)

Li Y, Adur MK, Kannan A et al. Progesterone Alleviates Endometriosis via Inhibition of Uterine Cell Proliferation, Inflammation and Angiogenesis in an Immunocompetent Mouse Model. PLoS One 2016-10-24 [PMID: 27776183] (Mouse)

Klein R, Stiller S, Gashaw I. Epidermal growth factor upregulates endometrial CYR61 expression via activation of the JAK2/STAT3 pathway Reprod Fertil Dev. 2012-01-01 [PMID: 22401280] (ICC/IF, Rabbit)

Xie JJ, Xu LY, Xie YM. Roles of ezrin in the growth and invasiveness of esophageal squamous carcinoma cells. Int J Cancer. 2009-06-01 [PMID: 19165868] (WB, IHC-P, Human)

Xie JJ, Xu LY, Wu JY et al. Involvement of CYR61 and CTGF in the fascin-mediated proliferation and invasiveness of esophageal squamous cell carcinomas cells. Am J Pathol. 2010-02-01 [PMID: 20056838] (IHC-P, WB, Human)

More publications at http://www.novusbio.com/NB100-356

Procedures

Western Blot Protocol for Cyr61/CCN1 Antibody (NB100-356)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Immunocytochemistry/ Immunofluorescence Protocol for Cyr61/CCN1 Antibody (NB100-356)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for Cyr61/CCN1 Antibody (NB100-356)
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 all the time).

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 NB100-356

NBL1-09705	Cyr61/CCN1 Overexpression Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

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