Product Datasheet

CTGF/CCN2 Antibody - BSA Free
NB100-724

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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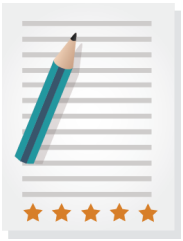
Updated 2/21/2025 v.20.1



NB100-724

CTGF/CCN2 Antibody - BSA Free

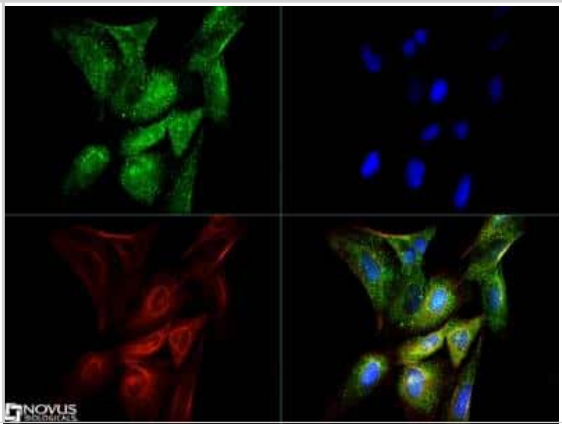
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	38 kDa

Product Description	
Host	Rabbit
Gene ID	1490
Gene Symbol	CCN2
Species	Human, Mouse, Rat
Immunogen	A synthetic peptide made to a C-terminal portion of human CTGF (between residues 299-349). [UniProt# P29279]

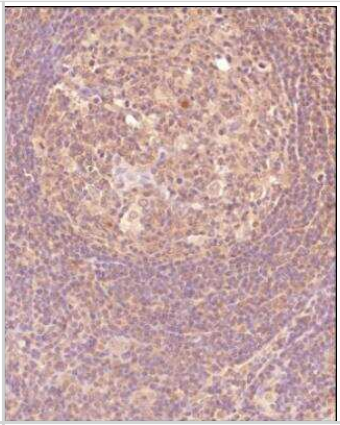
Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000 - 1:2000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:50 - 1:100, Immunohistochemistry-Paraffin reported in scientific literature (PMID 28562206)
Application Notes	In Western blot a band is observed at ~38 kDa. 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.

Images

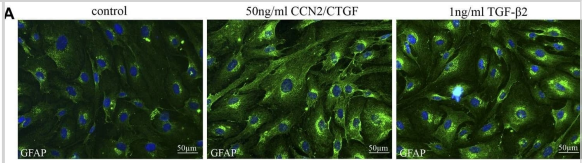
Immunocytochemistry/Immunofluorescence: CTGF/CCN2 Antibody [NB100-724] - CTGF antibody was tested in U2OS cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



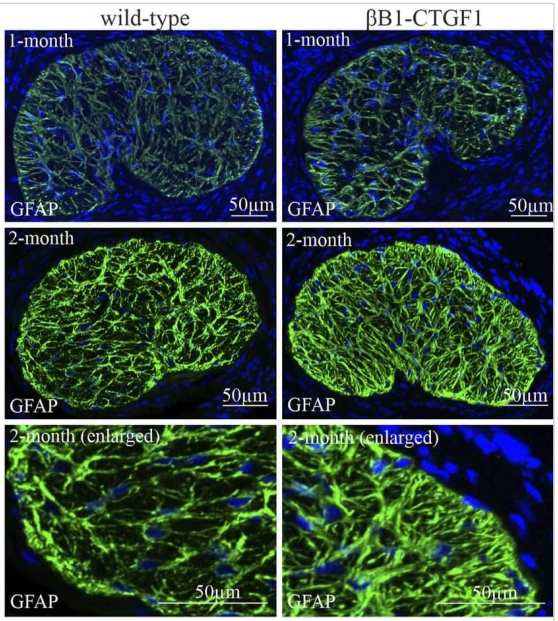
Immunohistochemistry-Paraffin: CTGF/CCN2 Antibody [NB100-724] - Analysis of a FFPE tissue section of human lymph node using 1:200 dilution of CTGF/CCN2 antibody (NB100-724). The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



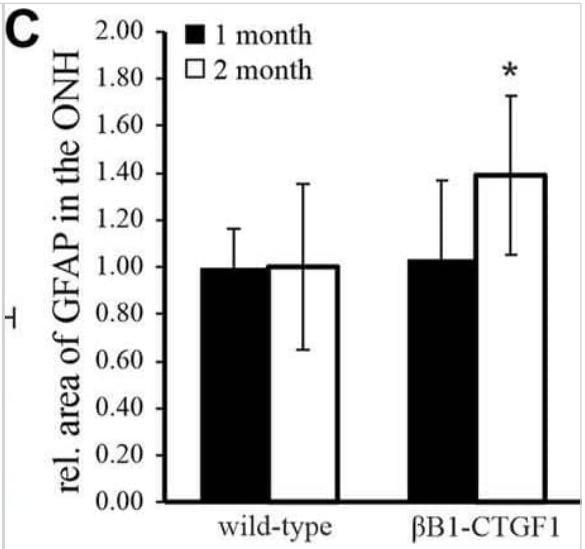
Immunocytochemistry/ Immunofluorescence: CTGF/CCN2 Antibody [NB100-724] - Analysis of astrocyte reactivity, the cytoskeleton & migration rate following treatment with TGF-β2 & CCN2/CTGF. (A) GFAP immunoreactivity (green) is increased in murine ON astrocytes following TGF-β2 & CCN2/CTGF treatment. Nuclei are stained with Dapi (blue) (B) Western Blot experiments analyses of α-actinin protein synthesis in murine ON astrocytes after the treatment with 1 ng/ml TGF-β2, 50 ng/ml CCN2/CTGF & 100 ng/ml CCN2/CTGF for 24 h. Densitometric analyses of Western Blot analyses shows the significant increase in α-actinin protein synthesis. Mean value of untreated cells (control) was set at 1. GAPDH was used to normalize protein intensity (n = 4; 50 ng CCN2/CTGF: *p = 0.029; 100 ng CCN2/CTGF: *p = 0.015; unpaired two-tailed t-test). (C) Scratch assay analyses show a significant increased migration rate for murine ON astrocytes after the treatment with ng/ml TGF-β2, 50 ng/ml CCN2/CTGF & 100 ng/ml CCN2/CTGF for 12 h. Mean value of migration rate of control cells was set to 100% (control: n = 15; TGF-β2: n = 12, **p = 0.008; 50 ng/ml CCN2/CTGF: n = 10, *p = 0.001; 100 ng/ml CCN2/CTGF: n = 4, **p = 0.0001). Data represented as mean ± SD. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35493079), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



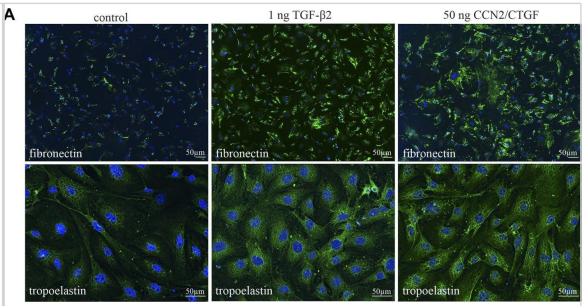
Immunohistochemistry: CTGF/CCN2 Antibody [NB100-724] - GFAP expression & synthesis in the ON & ONH in a murine glaucoma model. (A) GFAP immunoreactivity in the glial lamina of 1-month (green, upper panel) & 2 months (green, middle & lower panel) old βB1-CTGF1 mice compared to WT controls. Immunoreactivity of GFAP not altered in 1-month old TG & WT animals (green, upper panel). 2 month old βB1-CTGF1 mice showed an increased GFAP immunoreactivity compared to WT control (green, middle panel). Lower panel shows a magnified detail of the middle panel. Nuclei are stained w/ Dapi (blue). (B) Schmatic illustration of the optic nerve. A depicts region in the glial lamina where cross sections obtained. ONH (unmyelinated part) & ON (myelinated part) used for molecular analysis. (C) Quantification of immunohistochemical staining of GFAP in the glial lamina is not altered in 1 month old TG & WT animals (WT: n = 5; TG: n = 4). GFAP immunoreactivity is increased in 2 month old TG animals compared to WT littermates (WT: n = 10, TG: n = 13; *p = 0.039; two-tailed t-test compared to oretical mean of 1 (normalized control)). Mean value of WT animals (control) set at 1. (D) RT-PCR analyses revealed no alteration in the Gfap mRNA expression in the ON of 1-month (WT: n = 7; TG: n = 5) & 2 month old βB1-CTGF1 mice (WT: n = 15; TG: n = 16) compared to WT controls. In the ONH the Gfap mRNA expression is increased in the 2 month old TG compared to WT animals (WT: n = 6, TG: n = 5; *p = 0.04). The mRNA expression of Gfap is not altered in 1 month old animals (WT: n = 6, TG: n = 5). Mean value of WT animals (control) set at 1. RPL32 used to normalize mRNA expression. Data represented as mean ± SEM. PRL, Prelaminar region; PSL, Postlaminar region; ON, optic nerve; ONH, optic nerve head. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35493079), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



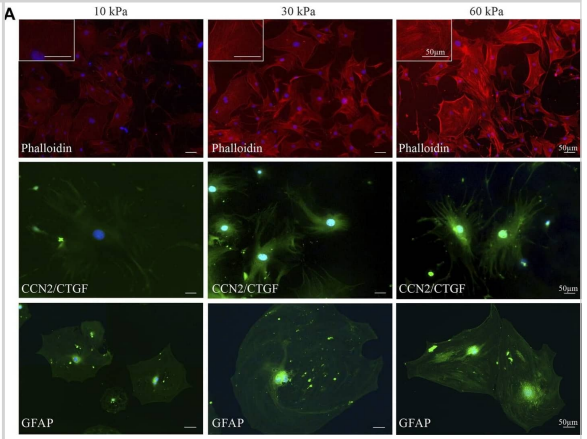
Immunohistochemistry: CTGF/CCN2 Antibody [NB100-724] - GFAP expression & synthesis in the ON & ONH in a murine glaucoma model. (A) GFAP immunoreactivity in the glial lamina of 1-month (green, upper panel) & 2 months (green, middle & lower panel) old βB1-CTGF1 mice compared to WT controls. Immunoreactivity of GFAP not altered in 1-month old TG & WT animals (green, upper panel). 2 month old βB1-CTGF1 mice showed an increased GFAP immunoreactivity compared to WT control (green, middle panel). Lower panel shows a magnified detail of the middle panel. Nuclei are stained w/ Dapi (blue). (B) Schmatic illustration of the optic nerve. A depicts region in the glial lamina where cross sections obtained. ONH (unmyelinated part) & ON (myelinated part) used for molecular analysis. (C) Quantification of immunohistochemical staining of GFAP in the glial lamina is not altered in 1 month old TG & WT animals (WT: n = 5; TG: n = 4). GFAP immunoreactivity is increased in 2 month old TG animals compared to WT littermates (WT: n = 10, TG: n = 13; *p = 0.039; two-tailed t-test compared to oretical mean of 1 (normalized control)). Mean value of WT animals (control) set at 1. (D) RT-PCR analyses revealed no alteration in the Gfap mRNA expression in the ON of 1-month (WT: n = 7; TG: n = 5) & 2 month old βB1-CTGF1 mice (WT: n = 15; TG: n = 16) compared to WT controls. In the ONH the Gfap mRNA expression is increased in the 2 month old TG compared to WT animals (WT: n = 6, TG: n = 5; *p = 0.04). The mRNA expression of Gfap is not altered in 1 month old animals (WT: n = 6, TG: n = 5). Mean value of WT animals (control) set at 1. RPL32 used to normalize mRNA expression. Data represented as mean ± SEM. PRL, Prelaminar region; PSL, Postlaminar region; ON, optic nerve; ONH, optic nerve head. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35493079), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: CTGF/CCN2 Antibody [NB100-724] - Analyses of ECM components in murine ON astrocytes following treatment w/ TGF-β2 & CCN2/CTGF. (A) Immunocytochemical staining against fibronectin (green, upper panel) showed a markedly increase following the treatment w/ 1 ng/ml TGF-β2 or 50 ng/ml CCN2/CTGF. Immunocytochemical staining against tropoelastin (green, lower panel) showed a pronounced increase after treatment w/ 1 ng/ml TGF-β2 or 50 ng/ml CCN2/CTGF. Nuclei stained w/ Dapi (blue). (B) Real-time RT-PCR analyses shows an intense upregulation of collagen 3α1, tropoelastin & fibronectin mRNA in murine ON astrocytes after treatment w/ 1 ng/ml TGF-β2, 50 ng/ml CCN2/CTGF or 100 ng/ml CCN2/CTGF for 24 h compared to untreated control cells (collagen 3α1: n = 3; TGF-β2 ***p = 0.0000004, 50 ngCTGF **p = 0.005, 100ngCTGF **p = 0.008; fibronectin: n = 6, TGF-β2 *p = 0.00004, 50 ng CTGF *p = 0.016, 100 ngCTGF *p = 0.05; elastin: n = 5, TGF-β2 *p = 0.026, 50 ng CTGF *0.04, 100 ng CTGF *p = 0.03). mRNA expression normalized to Gnb2l & mean value of control cells set at 1. (C) Western Blot analysis show an increase in protein synthesis of collagen 3α1, elastin & fibronectin in murine ON astrocytes after treatment w/ 1 ng/ml TGF-β2, 50 ng/ml CCN2/CTGF or 100 ng/ml CCN2/CTGF for 24 h compared to untreated control cells (collagen 3α1: n = 3, TGF-β2 *p = 0.03, 100 ngCTGF **p = 0.009; fibronectin: n = 5, **p = 0.005, 50 ngCTGF **p = 0.006, 100 ng CTGF *p = 0.02; tropoelastin: n = 5, TGF-β2 ***p = 0.0004, 50 ngCTGF **p = 0.002, 100 ngCTGF ***p = 0.00001). GAPDH & α-tubulin used to normalize protein synthesis & mean value of control cells set at 1. Right panel shows representative Western Blots for all three proteins. Data represented as mean ± SD. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35493079), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunocytochemistry/ Immunofluorescence: CTGF/CCN2 Antibody [NB100-724] - Increasing substratum stiffness causes alterations in murine ON astrocyte reactivity, actin cytoskeleton & CCN2/CTGF level. (A) Filamentous actin labeled by phalloidin (red, upper panel) is increased when murine ON astrocytes were cultured on substrates with higher stiffness (30 kPa, 60 kPa) compared to softer control (10 kPa). Increasing substratum stiffness leads to an enhanced formation of actin stress fibers. Immunoreactivity of CCN2/CTGF (green, middle panel) is markedly increased when murine ON astrocytes were grown on substrates with higher stiffness (30, 60 kPa) compared to softer control (10 kPa). Murine ON astrocytes show an enhanced GFAP protein synthesis (green, lower panel) when cultured on 30 or 60 kPa compared to 10 kPa. Nuclei were stained with Dapi (blue). (B) CCN2/CTGF protein synthesis is significantly increased in murine ON astrocytes grown on 60 kPa compared to 10 kPa (10 kPa: n = 5; 30 kPa: n = 5; 60 kPa: n = 5; *p = 0.04; unpaired two-tailed t-test). (C) Protein synthesis of GFAP (10 kPa: n = 6; 30 kPa: n = 6, **p = 0.005; 60 kPa: n = 4, *p = 0.04; unpaired two-tailed t-test) show an enhanced reactivity of murine ON astrocytes cultured on 30 kPa or 60 kPa compared to 10 kPa control. (D) Vimentin protein synthesis is significantly increased in murine ON astrocytes cultured on 60 kPa compared to 10 kPa (10 kPa: n = 4; 30 kPa: n = 4; 60 kPa: n = 3 ***p = 0.00002). Protein synthesis was normalized to α-tubulin & mean value of control (10 kPa) was set at 1. Data represented as mean ± SEM. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35493079), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Dillinger AE, Weber GR, Mayer M, Schneider M et Al. CCN2/CTGF-A Modulator of the Optic Nerve Head Astrocyte Front Cell Dev Biol 2022-05-02 [PMID: 35493079]

Cho S, Roh K, Park J et al. Hydrolysis of Hyaluronic Acid in Lymphedematous Tissue Alleviates Fibrogenesis via TH1 Cell-Mediated Cytokine Expression. Sci Rep 2017-12-01 [PMID: 28232732]

Laura J. Brylka, Assil-Ramin Alimy, Miriam E. A. Tschaffon-Müller, Shan Jiang, Tobias Malte Ballhause, Anke Baranowsky, Simon von Kroge, Julian Delsmann, Eva Pawlus, Kian Eghbalian, Klaus Püschel, Astrid Schoppa, Melanie Haffner-Luntzer, David J. Beech, Frank Timo Beil, Michael Amling, Johannes Keller, Anita Ignatius, Timur A. Yorgan, Tim Rolvien, Thorsten Schinke Piezo1 expression in chondrocytes controls endochondral ossification and osteoarthritis development Bone Research 2024-02-23 [PMID: 38395992]

Huang, HC;Wang, TY;Rousseau, J;Mungaray, M;Michaud, C;Plaisier, C;Chen, ZB;Wang, KC; Lesion-specific suppression of YAP/TAZ by biomimetic nanodrug ameliorates atherosclerosis development bioRxiv : the preprint server for biology 2023-04-26 [PMID: 37163067] (Western Blot, Mouse)

Buse J Einfluss einer ERK1/2-Resistenz auf Zellwachstum und Invasivit□t von triple-negativen Mammakarzinomzellen Thesis 2023-01-27

Hazra R, Brine L, Garcia L et al. Platr4 is an early embryonic lncRNA that exerts its function downstream on cardiogenic mesodermal lineage commitment Developmental cell 2022-11-07 [PMID: 36347239] (RIP, Mouse)

Hellinger JW, SchOmel F, Buse JV et al. Identification of drivers of breast cancer invasion by secretome analysis: insight into CTGF signaling Sci Rep 2020-10-21 [PMID: 33087801] (IF/IHC, ICC/IF, Human)

Wu SH, Lu IC, Tai MH et al. Erythropoietin Alleviates Burn-induced Muscle Wasting Int J Med Sci 2020-01-01 [PMID: 31929736] (WB, Rat)

Ho LTY, Osterwald A, Ruf I et al. Role of the autotaxin-lysophosphatidic acid axis in glaucoma, aqueous humor drainage and fibrogenic activity Biochim Biophys Acta Mol Basis Dis 2019-10-21 [PMID: 31648019] (WB, Human)

Pan X, Jing Y, Liu W et al. Lipopolysaccharide induces the differentiation of hepatic progenitor cells into myofibroblasts via activation of the Hedgehog signalling pathway. Cell Cycle. 2017-05-31 [PMID: 28562206]

Zhu L, Zhao S, Liu S et al. PTEN Regulates Renal Extracellular Matrix Deposit via Increased CTGF in Diabetes Mellitus J Cell Biochem [PMID: 26447680]

Yijing L, Liu H, Yuan C et al. The effects of qindan-capsule-containing serum on the TGF-beta1/ERK signaling pathway, matrix metalloproteinase synthesis and cell function in adventitial fibroblasts. Pharm Biol 2013-02-04 [PMID: 23373709] (WB, Rat)

More publications at http://www.novusbio.com/NB100-724

Procedures

Western Blot Protocol for CTGF Antibody (NB100-724)
CTGF/CCN2 Antibody:
Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 20 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS for 1 hour at RT.
6. Rinse membrane once in TBS and then wash 3x 10 minutes.
7. Dilute the rabbit anti-CTGF primary antibody (NB 100-724) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse membrane once in TBS and then wash 3x 10 minutes.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Rinse membrane once in TBS and then wash 3x 10 minutes.
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce's ECL is the standard reagent used at for this Novus Biologicals assay).

Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunocytochemistry/Immunofluorescence Protocol for CTGF Antibody (NB100-724)
CTGF/CCN2 Antibody:
Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry-Paraffin Protocol for CTGF/CCN2 Antibody (NB100-724)
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 NB100-724

NB820-59661	Mouse Kidney Whole Tissue Lysate (Adult Whole Normal)
NB100-724PEP	CTGF/CCN2 Antibody Blocking Peptide
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
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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