Product Datasheet

Cyr61/CCN1 Antibody (3A7.1B8) - BSA Free
NBP2-36490

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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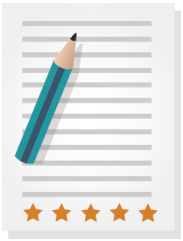
Updated 10/23/2024 v.20.1



NBP2-36490

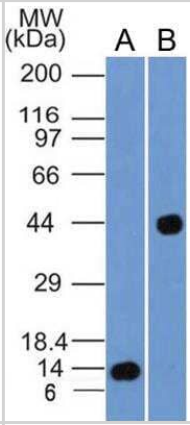
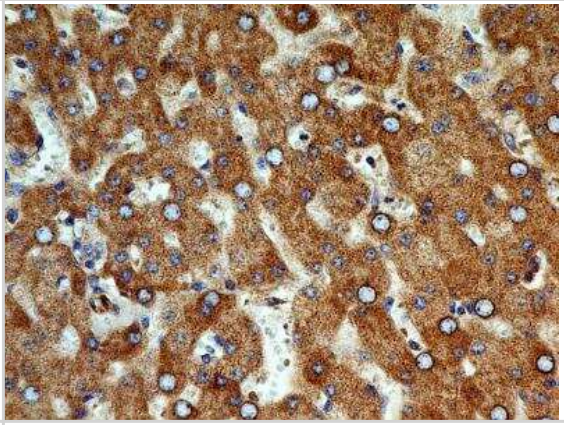
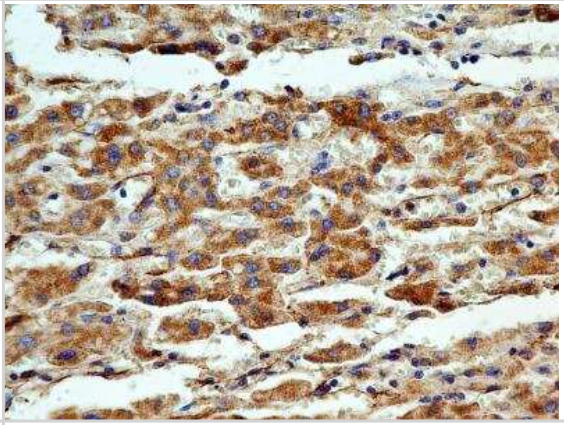
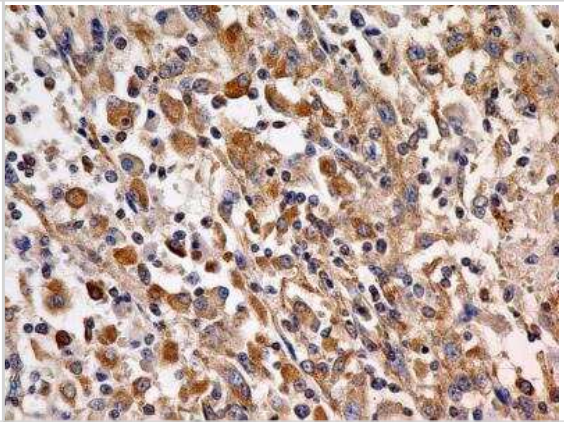
Cyr61/CCN1 Antibody (3A7.1B8) - BSA Free

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	3A7.1B8
Preservative	0.02% Sodium Azide
Isotype	IgG2b Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	3491
Gene Symbol	CCN1
Species	Human
Reactivity Notes	Immunogen's sequence similarity with other species: Rat (81%), Mouse (79%), Camel (88%), Naked Mole Rat (87%), Porcine (80%), Bovine / Sheep / Horse (82%), Chicken (63%)
Immunogen	Partial recombinant protein from human Cyr61/CCN1 protein (between residues 100-300). [Swiss-Prot O00622]

Product Application Details	
Applications	Western Blot, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 4 ug/ml, Immunohistochemistry 5 ug/ml, Immunohistochemistry-Paraffin 5 ug/ml
Application Notes	Protein CYR61 is a secreted protein and its expected immunolocalization would be cytoplasmic as well as the extracellular spaces.

Images

Western Blot: Cyr61/CCN1 Antibody (3A7.1B8) [NBP2-36490] - WB analysis of (A) 12kDa Partial Recombinant Cyr61/CCN1 protein and (B) Human Liver lysate using Cyr61/CCN1 antibody (clone 3A7.1B8) at 4ug/ml concentration.	
Immunohistochemistry-Paraffin: Cyr61/CCN1 Antibody (3A7.1B8) [NBP2-36490] - IHC analysis of formalin-fixed paraffin-embedded tissue section of human liver using Cyr61/CCN1 antibody (clone 3A7.1B8) at 5 ug/ml concentration. The hepatocytes generated a specific cytoplasmic staining with no signal in the cellular nuclei or the sinusoids.	
Immunohistochemistry-Paraffin: Cyr61/CCN1 Antibody (3A7.1B8) [NBP2-36490] - IHC analysis of formalin-fixed paraffin-embedded tissue section of human hepatocellular carcinoma using Cyr61/CCN1 antibody (clone 3A7.1B8) at 5 ug/ml concentration. The cancer cells generated a specific cytoplasmic staining with some staining in the inter-cellular spaces but no signal in the cellular nuclei, tumor stroma or the RBCs.	
Immunohistochemistry-Paraffin: Cyr61/CCN1 Antibody (3A7.1B8) [NBP2-36490] - IHC analysis of formalin-fixed paraffin-embedded tissue section of human Renal Cell Carcinoma using Cyr61/CCN1 antibody (clone 3A7.1B8) at 5 ug/ml concentration. The cancer cells generated a specific cytoplasmic staining with some staining in the inter-cellular spaces but no signal in the cellular nuclei, tumor stroma or the RBCs.	

Procedures

Immunohistochemistry-Paraffin protocol for Cyr61/CCN1 Antibody (NBP2-36490)

Cyr61/CCN1 Antibody (3A7.1B8):

1. Deparaffinize the tissue sections by immersing the slides in Xylene with two changes for 10 min each. Sections should not get dried at any stage from this point.
2. Rehydrate the tissue sections by immersing the slides in decreasing grades of ethanol as follows:
 a. Immerse in 100% ethanol with 2 changes for 5 minutes each
 b. Immerse in 95% ethanol with 2 changes for 5 minutes each
 c. Immerse in 90% ethanol for 5 minutes
 d. Immerse in 70% ethanol for 5 minutes
 e. Immerse in 50% ethanol for 5 minutes
 f. Immerse in distilled water for 5 minutes
3. Antigen Retrieval (Microwave Method):
 a. Immerse the slides in a microwave compatible tray containing 10 mM Sodium Citrate buffer (pH 6.0) with 0.05% Tween 20.
 b. Boil the slides and maintain the sub-boiling temperature for 5 minutes in the microwave. Thereafter, take out the tray very carefully and cool it at room temperature (RT) for about 30 minutes.
 c. Wash the slides 3 times, 3 minutes each by immersing them in TBST (Tris Buffered Saline having 0.05% Tween 20).
4. Quenching of Endogenous Peroxidase:
 a. Incubate the slides in 3% hydrogen peroxide prepared in methanol for 15 minutes (at RT, in dark conditions).
 b. Wash the slides in TBST 3 times, 3 minutes each.
5. Protein Blocking:
 a. Incubate the sections with background sniper solution at RT for 15 minutes (Biocare Medicals, USA).
 b. Wash the sections 3 times, 3 min each by immersing the slides in TBST.
6. Primary Antibody:
 a. Dilute the primary antibody at 5ug/ml concentration using PBS as a diluent.
 b. Incubate the sections with diluted primary antibody for 90 minutes at RT in a humidified chamber.
 c. Thereafter, wash the slides 4 times, 5 minutes each with TBST.
7. Probe (Secondary Reagent):
 a. Incubate with MACH 1 Mouse probe for 15 minutes at RT.
 b. Incubate for 30 min at room temperature with HRP-Polymer (Biocare Medical, USA).
 c. Wash the slides with TBST 4 times, 5 minutes each
8. Chromogen:
 a. Mix 32ul of DAB Chromogen with 1 ml of DAB substrate buffer (Biocare Medical, USA).
 b. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds - 5 minutes).
 c. As soon as an appropriate color develops, rinse the slides with deionized water (2-3 brief rinses).
9. Counter stain:
 a. Counter stain with Hematoxylin for 30 seconds (Vector Labs, USA).
 b. Wash in deionized water for 1-2 minutes to clear the extra stain.
 c. Incubate the slides in bluing solution or Scott's water twice for 2 minutes each time.
10. Dehydrate the sections in increasing grades of alcohols:
 a. 50% alcohol for 1 minute
 b. 70% for 1 minute
 c. 90% for 1 minute
 d. 95% for 1 minute
 e. 100% for 1 minute
 f. Xylene with 2 changes for 2 minutes each
11. Mount with DPX mount and cover-slip glass (Fisher Scientific, USA), carefully not allowing any air bubbles to enter.

NOTE:- This protocol is provided as a reference tool only. Depending upon the type of tissues /tissue processing and reagents employed, the end user will need to optimize the final conditions for achieving an expected staining.

Western Blot protocol for Cyr61/CCN1 Antibody (NBP2-36490)
Cyr61/CCN1 Antibody (3A7.1B8):
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute anti-Cyr61/CCN1 (3A7.1B8) primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer 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.
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

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NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43317-0.5mg	Mouse IgG2b Kappa Light Chain Isotype Control (MG2b)
NBP2-36490B	Cyr61/CCN1 Antibody (3A7.1B8) [Biotin]

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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