Product Datasheet

TSLPR/CRLF2 Antibody
NBP2-29613

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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www.novusbio.com/NBP2-29613

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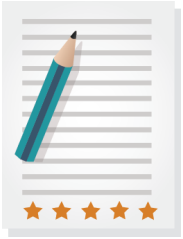


NBP2-29613

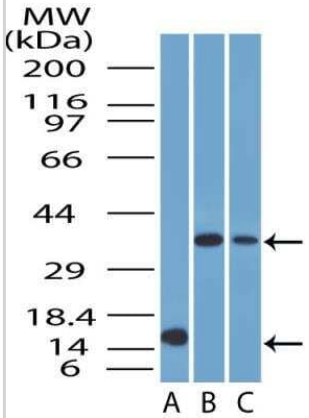
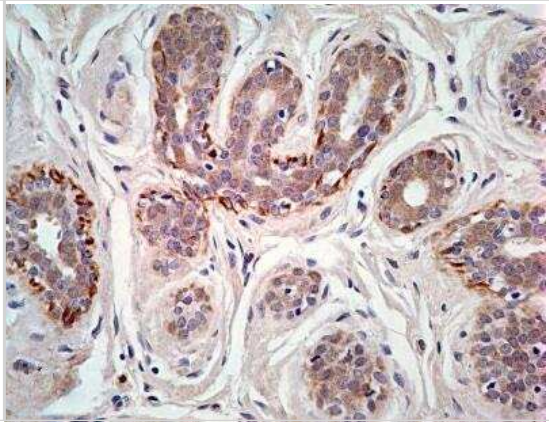
TSLPR/CRLF2 Antibody

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	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Protein A purified
Buffer	PBS
Target Molecular Weight	42 kDa

Product Description	
Host	Rabbit
Gene ID	64109
Gene Symbol	CRLF2
Species	Human
Reactivity Notes	Human
Immunogen	Partial recombinant protein made to an internal portion of human CRLF2 protein (between residues 100-300) [NCBI AF338733_1]

Product Application Details	
Applications	Western Blot, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1.0 ug/ml, Immunohistochemistry 5 ug/ml, Immunohistochemistry-Paraffin 5 ug/ml
Application Notes	CRLF2 is a 371 AA long protein (42 kDa) which undergoes processing via removal of signal peptide (n-terminal 22 AA) through cleavage at AA 22, and the cleaved CRLF2 protein (349 AA) undergoes additional modifications such as glycosylation and disulfide bond formation. In our WB testing, single specific bands were observed at ~35-38 kDa position (potentially unglycosylated form) in human skeletal muscle and liver lysates. The glycosylated monomers of CRLF2 may run at ~50-60kDa position, whereas, under the non-reducing conditions, additional bands representing its dimeric/heterodimeric forms may be seen at ~120/170 kDa position (PMIDs 22915648, 21631431, 20018760, 22915648). 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

Western Blot: CRLF2 Antibody [NBP2-29613] - Western blot analysis of CRLF2 antibody in 1. partial recombinant CRLF2 protein 2. Human skeletal muscle lysate and 3. Human liver lysate	
Immunohistochemistry-Paraffin: CRLF2 Antibody [NBP2-29613] - IHC-P analysis of CRLF2 protein in a tissue section of esophageal squamous cell carcinoma using CRLF2 antibody at a concentration of 5 ug/ml. The representative image shows a well-differentiated squamous area with distinct squamous pearl or keratin pearl formation which developed intense staining.	
Immunohistochemistry-Paraffin: CRLF2 Antibody [NBP2-29613] - IHC-P analysis of CRLF2 protein in a section of normal breast tissue using CRLF2 antibody at a concentration of 5 ug/ml. The breast ductal or acinar epithelial cells showed moderate expression whereas the myoepithelial cells depicted very strong CRLF2 positivity. The interlobular stroma/connective tissue showed weak to negligible staining.	

Procedures

Western Blot Protocol for CRLF2 Antibody (NBP2-29613)
TSLPR/CRLF2 Antibody: https://www.novusbio.com/products/tslpr-crlf2-antibody_nbp2-29613
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-CRLF2 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%.

Immunohistochemistry-Paraffin Protocol for CRLF2 Antibody (NBP2-29613)

TSLPR/CRLF2 Antibody: https://www.novusbio.com/products/tslpr-crlf2-antibody_nbp2-29613

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.

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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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