Product Datasheet

CD74 Antibody (PIN.1) - BSA Free
NB100-1985

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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Publications: 6

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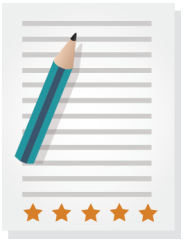
Updated 10/23/2024 v.20.1



NB100-1985

CD74 Antibody (PIN.1) - BSA Free

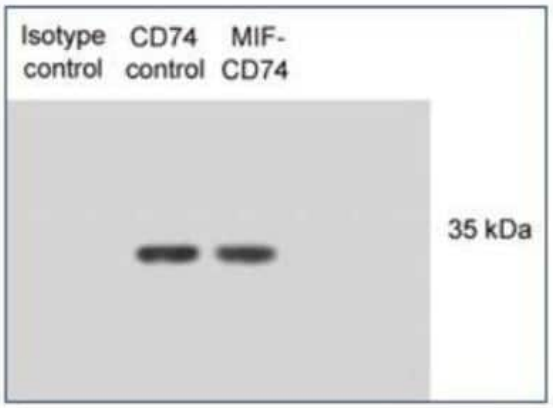
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	PIN.1
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	972
Gene Symbol	CD74
Species	Human, Mouse (Negative)
Reactivity Notes	Human. Does not react with mouse.
Specificity/Sensitivity	This detects an ~33-35 kDa protein doublet, corresponding to the apparent molecular mass of the p33 and p35 forms of human CD74.
Immunogen	Peptide corresponding to residues 12-27 of CD74, human invariant chain (short form).

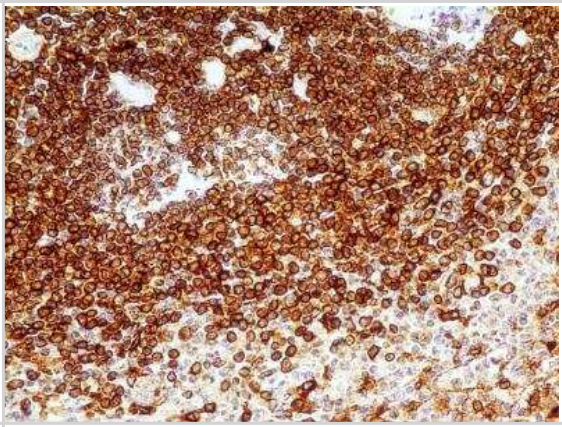
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, CyTOF-ready
Recommended Dilutions	Western Blot 1 ug/ml, Simple Western, Flow Cytometry 1 ug/million cells, Immunohistochemistry 5 ug/ml, Immunocytochemistry/ Immunofluorescence 1:10 -1:2000, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 5 ug/ml, CyTOF-ready
Application Notes	In Simple Western only 10-15 ul of the recommended dilution is used per data point. In WB this detects an ~33-35 kDa protein doublet, corresponding to the apparent molecular mass of the p33 and p35 forms of human CD74. This antibody is CyTOF ready. See Simple Western Antibody Database for Simple Western validation: tested in Jurkat lysate (0.2 mg/ml); antibody dilution of 1:400; separated by size; detects a band at 62 kDa

Images

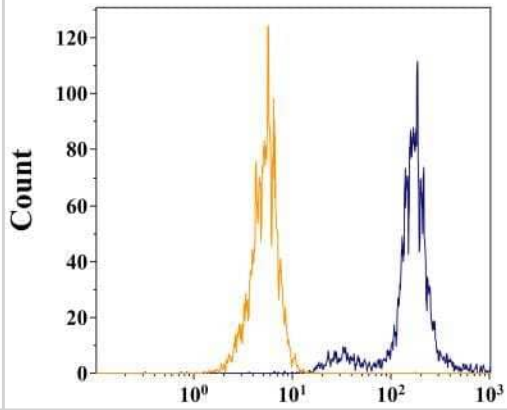
Western Blot: CD74 Antibody (PIN.1) [NB100-1985] - Western blot of CD74 from IP N87 lysates mixed with macrophage migration inhibitory factor (MIF).



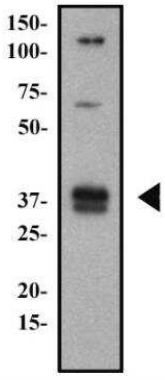
Immunohistochemistry-Paraffin: CD74 Antibody (PIN.1) [NB100-1985] - IHC analysis of formalin-fixed paraffin-embedded tissue section of human spleen using mouse monoclonal CD74 antibody (clone PIN.1) at 5 ug/ml concentration.

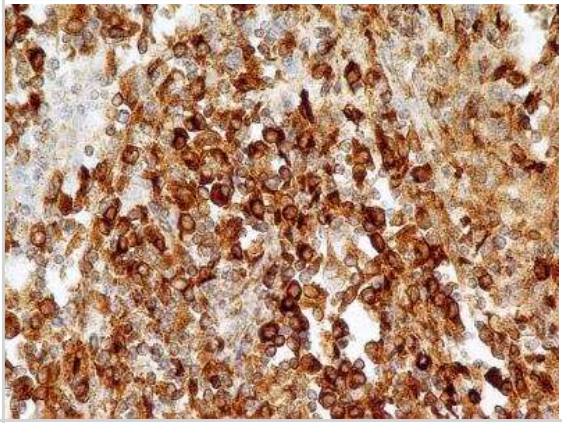
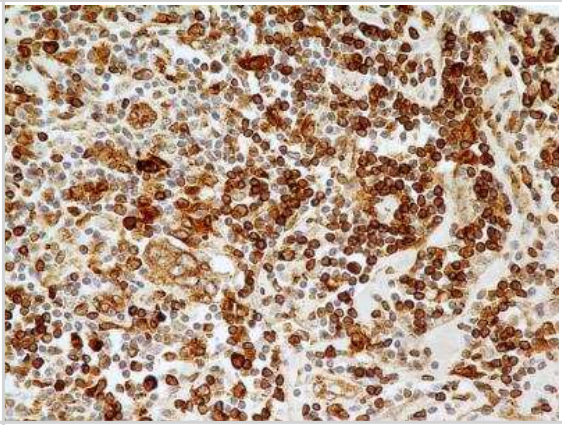


Flow Cytometry: CD74 Antibody (PIN.1) [NB100-1985] - CD74 in human PBMCs (gated on all monocytes). Orange represents isotype control and blue represents anti-CD74 antibody staining.



Western Blot: CD74 Antibody (PIN.1) [NB100-1985] - Ramos whole cell protein was separated on a 4-12% gel by SDS-PAGE and protein transferred to PVDF. The membrane was probed with anti-CD74 at 2 ug/ml and detected with and anti-mouse HRP secondary antibody using chemiluminescence. The arrow head shows the detection of CD74.



Immunohistochemistry-Paraffin: CD74 Antibody (PIN.1) [NB100-1985] - IHC analysis of formalin-fixed paraffin-embedded tissue section of human renal cell carcinoma using mouse monoclonal CD74 antibody (clone PIN.1) at 5 ug/ml concentration.	
Immunohistochemistry-Paraffin: CD74 Antibody (PIN.1) [NB100-1985] - IHC analysis of formalin-fixed paraffin-embedded tissue section of human pulmonary squamous cell carcinoma using mouse monoclonal CD74 antibody (clone PIN.1) at 5 ug/ml concentration.	
CD74 (PIN.1) was detected in immersion fixed U-2 OS human osteosarcoma cell line using Mouse anti-CD74 (PIN.1) Protein G Purified Monoclonal Antibody conjugated to Alexa Fluor® 647 (Catalog # NB100-1985AF647) (light blue) at 10 µg/mL overnight at 4C. Cells were counterstained with DAPI (dark blue). Cells were imaged using a 100X objective and digitally deconvolved.	

Publications

Koh YW, Han JH, Haam S, Lee HW. An immune-related gene expression signature predicts brain metastasis in lung adenocarcinoma patients after surgery: gene expression profile and immunohistochemical analyses Translational Lung Cancer Research 2021-03-15 [PMID: 33718023] (Immunohistochemistry)

Weng Y, Lou J, Bao Y et al. Single-Cell RNA Sequencing Technology Revealed the Pivotal Role of Fibroblast Heterogeneity in Angiotensin II-Induced Abdominal Aortic Aneurysms DNA and cell biology 2022-04-22 [PMID: 35451888]

Tanese K, Hashimoto Y, Berkova Z et al. Cell Surface CD74-MIF Interactions Drive Melanoma Survival in Response to Interferon-Gamma J. Invest. Dermatol. 2015-06-03 [PMID: 26039541] (WB, Human)

Lamb CA, Cresswell P. Assembly and transport properties of invariant chain trimers and HLA-DR-invariant chain complexes. J Immunol. 1992-06-01 [PMID: 1588042]

Roche PA, Marks MS, Cresswell P. Formation of a nine-subunit complex by HLA class II glycoproteins and the invariant chain. Nature. 1991-12-05 [PMID: 1956401]

Katsel P, Tan W, Haroutunian V. Gain in brain immunity in the oldest-old differentiates cognitively normal from demented individuals. PLoS One 4(10):e7642. 2009-10-29 [PMID: 19865478]

Procedures

Immunohistochemistry-Paraffin protocol for CD74 Antibody (NB100-1985)
CD74 Antibody (PIN.1):
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).
a. Apply 200ul DAB mixture/section and incubate at RT in dark conditions (few seconds - 5 minutes).
b. 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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HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NB100-1985AF647	CD74 Antibody (PIN.1) [Alexa Fluor® 647]

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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