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Datasheet for 200-301-A88

Mesothelin Antibody

Overview

Description:	Anti-Mesothelin (MOUSE) Monoclonal Antibody - 200-301-A88
Item No.:	200-301-A88
Size:	100 µg
Applications:	FC, IHC, WB
Reactivity:	Human
Host Species:	Mouse

Product Details

Background:	Anti Mesothelin Antibody recognizes Mesothelin that is a glycosyl-phosphatidylinositol–anchored glycoprotein present on the cell surface of various human solid tumors. The mesothelin (MSLN) gene encodes a 71-kDa precursor protein that is processed to a 40-kDa glycosylphosphatidylinositol–anchored protein that composes the mature portion and an NH2 terminal 31-kDa fragment called megakaryocyte-potentiating factor that is released from the cell. Mesothelin is a tumor differentiation antigen present at low levels on a restricted set of normal adult tissues, such as mesothelium, but aberrantly over expressed in mesotheliomas, ovarian, and pancreatic cancers. The biological functions of mesothelin remain elusive. A recent study showed that mesothelin binds to MUC16/CA125, and that this interaction mediates cell adhesion, suggesting that there may be an important role for MUC16/CA125 and mesothelin in the metastatic spread of ovarian cancer.
Synonyms:	mouse anti-Mesothelin Antibody, Mesothelian, MN, MB, Pre-pro-megakaryocyte-potentiating factor, CAK1 antigen
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	MN-1
Format:	IgG2a

Target Details

Gene Name:	MSLN

Reactivity:	Human
Immunogen Type:	Recombinant Protein
Immunogen:	This antibody was produced in mesothelin-deficient mice by immunizations with plasmid cDNA encoding human MSLN full length protein followed by a single boost of a recombinant human mesothelin-Fc fusion protein.
Purity/Specificity:	This antibody is directed against human mesothelin protein. This product was purified from tissue culture supernatant fluid by Protein A chromatography. Cross reactivity with homologues from other sources has not been tested.
Relevant Links:	• UniProtKB - Q13421 • NCBI - NP_005814 • GeneID - 10232

Application Details

Tested Applications:	FC, IHC, WB
Application Note:	This antibody has been tested for use in immunohistochemistry, flow cytometry, and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 40 kDa in size corresponding to mature mesothelin by western blotting in the appropriate cell lysate or extract. For immunohistochemistry, archival PEFF human tissues were deparaffinized followed by hydration. Antigen-retrieval is recommended. Block tissues with 1% BSA in PBS for 30 min at 23° C. Antibodies are diluted in 1% BSA and reacted with tissue for 60 min at room temperature.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:50,000
FC:	1:800
IHC:	1:100
WB:	1:1,000

Formulation

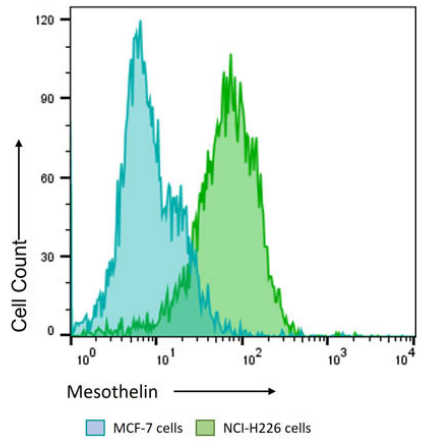
Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide

Stabilizer:	None

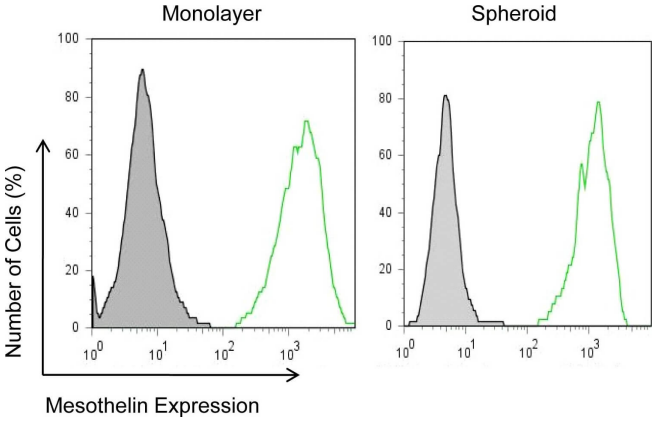
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

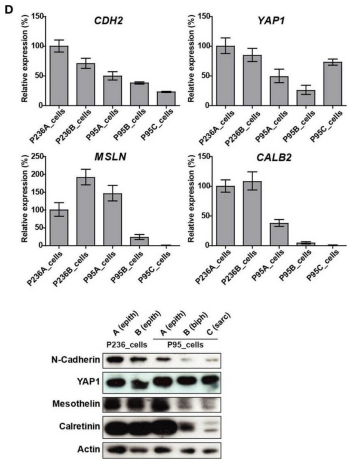
Images



Flow Cytometry
Flow Cytometry Results of Anti-Mesothelin (MOUSE) Monoclonal Antibody. The green histogram shows NCI-H226 cells and blue histogram shows MCF-7 cells. Both cell lines are stained with a 1:800 dilution Anti-Mesothelin (MOUSE) Monoclonal Antibody. The secondary antibody use was Anti-Mouse IgG (H&L) (GOAT) Antibody DyLight™ 488 Conjugated (p/n 610-141-002, lot#43322) at the 1:400 dilution.

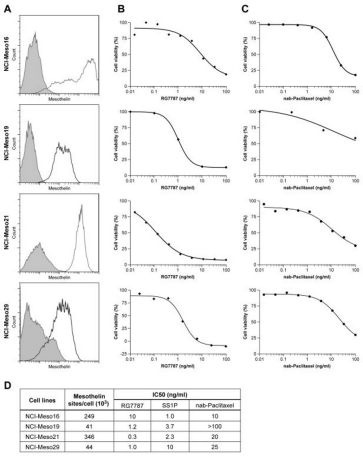


Flow Cytometry
Mesothelin expression in mesothelioma monolayers and spheroids.NCI-H226 cells incubated with an anti-mesothelin mAb (MN) and detected with goat anti-mouse IgG conjugated with Alexa488 by flow cytometry. Figure provided by CiteAb. Source: PLoS One, PMID: 21305058.



Western Blot

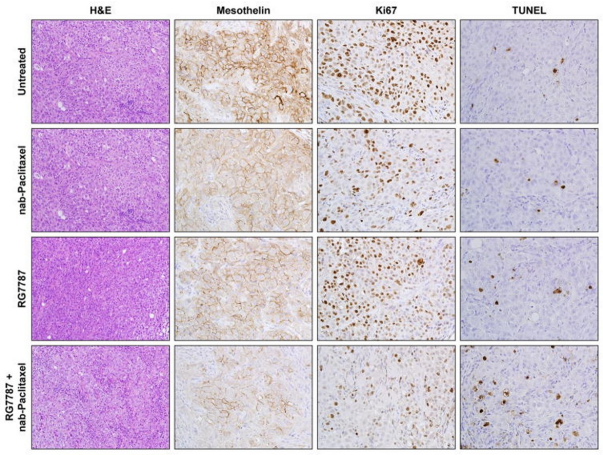
(A) Tumor processing flow-chart of 235 samples, which were processed for RNA extraction, cell culture and embedding either in PFA or OCT. Samples showing attributes which are grayed in the chart, were not used in the live cell biobank. (B) Circos whole genome copy number variations (CNVs) view of tumor and primary cell culture in patients malignant pleural mesothelioma (MPM) 236 and MPM95. The quilt plot highlights the CNV and SNVs in genes that are part of MPM landscape (2). (C) Immunofluorescence analysis of selected markers in primary culture from patient MPM236. Scale bar 200 µm. (D) Selected genes expression analysis at mRNA (upper panel) and protein (lower panel) level in primary cultures derived from samples from two patients, MPM236 and MPM95. The latter one underwent EMT during disease progression. (E) Selected genes expression analysis at mRNA in tumor samples from patients MPM236 and MPM95. (F) Significant correlation between gene expression changes in tumor and primary culture from patient MPM236 at passage 3. Figure provided by CiteAb.
Source: Front Oncol, PMID: 29527515.



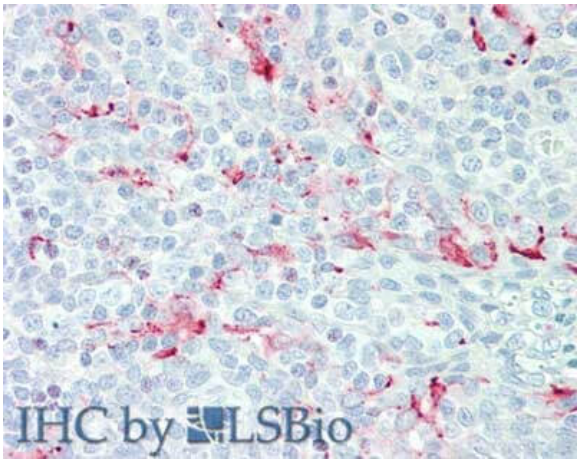
Cell lines	Mesothelin sites/cell (10^3)	IC50 (ng/ml)		
		RG7787	SS1P	nab-Paclitaxel
NCI-Meso16	249	10	1.0	10
NCI-Meso19	41	1.2	3.7	>100
NCI-Meso21	346	0.3	2.3	20
NCI-Meso29	44	1.0	10	25

Flow Cytometry

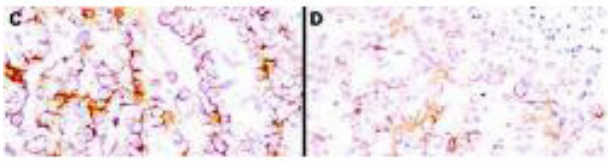
(A) Primary mesothelioma cell cultures were stained with anti-mesothelin antibody MN (p/n 200-301-A88) followed by staining with goat anti-mouse antibody conjugated with R-PE and the binding was analyzed by flow cytometry. Results are shown in terms of histogram plots for each cell line where open area depicts the binding of MN antibody and the gray area shows the binding of the isotope control antibody. (B) Cytotoxicity of RG7787 against the primary mesothelioma cell cultures. Eight thousand tumor cells were seeded in a 96-well plate and serial dilutions of RG7787 were added. After 4 days of incubation, cell viability was measured by WST-8 assay. (C) Cytotoxicity of nab-Paclitaxel against primary mesothelioma cells. Eight thousand tumor cells were seeded in a 96-well plate and serial dilutions of Nab-Paclitaxel were added. The cells were incubated for 4 days and the percent of viable cells were determined by WST-8 assay. (D) Table summarizing mesothelin expression in the primary mesothelioma cell lines as well as their sensitivity to the immunotoxins SS1P and RG7787 as well as nab-Paclitaxel.
Fig 1. PMID: 27635089



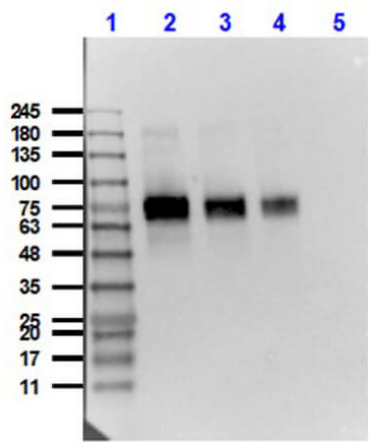
Immunohistochemistry
Mice were subcutaneously inoculated with 5x106 NCI-Meso29 cells and the treatment was started when tumors size reached 100 mm3. Mice were treated with no treatment; 1 cycle of 3x 2.5mg/kg RG7787 QOD i.v.; 1 cycle of 1 x 75 mg/kg nab-Paclitaxel and 1 cycle of 3 x 25mg/kg RG7787 combined with 1 cycle 1 x 75 mg/kg nab-Paclitaxel. Representative tumor tissues from different groups were harvested after the 1st cycle of the indicated treatments on Day 33, and representative H&E staining, IHC staining for MSLN and Ki-67 expression, as well as TUNEL staining with the paraffin sections of tumor tissues were shown. Scale bar in H&E, 1000 µm. Scale bar in MSLN, Ki67, TUNEL staining, 200 µm.
Fig 5. PMID: 27635089



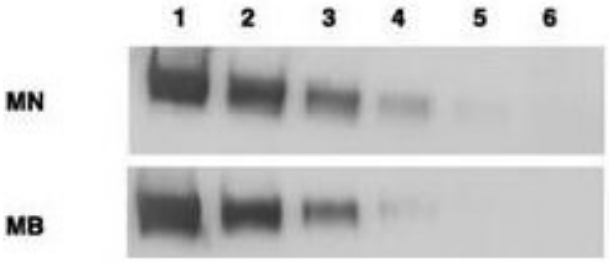
Immunohistochemistry
Immunohistochemistry of Mouse anti-Mesothelin antibody. Tissue: human tonsil. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: anti-Mesothelin antibody at 15 µg/mL for 1 h at RT. Secondary antibody: Peroxidase mouse secondary antibody at 1:10,000 for 45 min at RT. Staining: Mesothelin as precipitated red signal with hematoxylin purple nuclear counterstain.



Immunohistochemistry
Immunohistochemistry using Rockland's anti-mesothelin antibody to react with two epitopes on mesothelin in PEFF human mesothelioma tissue sections treated by antigen retrieval methods. Anti-mesothelin primary antibodies were used at 10 µg/mL to label these sections as follows: C, MAb MB; and D, MAb MN followed by goat anti-mouse IgG conjugated to horseradish peroxidase at 25 µg/mL in 1% BSA/PBS for 30 minutes. (magnification, ×200; bar, 50 µm). Reprinted with permission from Clin.Cancer Res. 11(16):5840 -6.



Western Blot
Western Blot of Mouse Anti-Mesothelin Antibody. Lane 1: Opal Prestained Molecular Weight Marker (p/n MB-210-0500). Lane 2: HeLa [10µg] + Mesothelin-Fc [0.1µg]. Lane 3: HeLa [10µg] + Mesothelin-Fc [0.05µg]. Lane 4: HeLa [10µg] + Mesothelin-Fc [0.02µg]. Lane 5: HeLa Whole Cell Lysate (p/n W09-000-364). Primary Antibody: Anti-Mesothelin at 1µg/mL overnight at 2-8°C. Secondary Antibody: Rabbit Anti-Mouse IgG HRP conjugated (p/n 610-4302) at 1:40,000 for 30 mins at RT. Block: BlockOut Buffer (p/n MB-073) 30 mins at RT. Exposure: 15 sec. Predicted MW: 40kDa Mesothelin + Fc region 30kDa. Observed MW: ~70-75kDa.



Western Blot
Western blotting using Rockland's anti-mesothelin antibodies to detect mesothelin-Fc at 100 ng (lane 1), 25 ng (lane 2), 6 ng (lane 3), 2 ng (lane 4) and 0.4 ng (lane 5). Lane 6 contains 50 ng of CDC25-Fc. Proteins were separated on 4-20% gradient gel by SDS-PAGE followed by transfer to PVDF membrane. Primary antibody was used at 1 µg/ml followed by reaction with ALP goat anti-mouse IgG and BCIP/NBT substrate. Reprinted with permission from Clin.Cancer Res. 11(16):5840-6.

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