

**Datasheet for 200-301-A88****Mesothelin Antibody****Overview**

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

**Product Details**

<b>Background:</b>	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.
<b>Synonyms:</b>	mouse anti-Mesothelin Antibody, Mesothelian, MN, MB, Pre-pro-megakaryocyte-potentiating factor, CAK1 antigen
<b>Host Species:</b>	Mouse
<b>Clonality:</b>	Monoclonal
<b>Clone ID:</b>	MN-1
<b>Format:</b>	IgG2a

**Target Details**

<b>Gene Name:</b>	MSLN
-------------------	------

<b>Reactivity:</b>	Human
<b>Immunogen Type:</b>	Recombinant Protein
<b>Immunogen:</b>	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.
<b>Purity/Specificity:</b>	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.
<b>Relevant Links:</b>	<ul style="list-style-type: none"><li>• <a href="#">UniProtKB - Q13421</a></li><li>• <a href="#">NCBI - NP_005814</a></li><li>• <a href="#">GeneID - 10232</a></li></ul>

## Application Details

<b>Tested Applications:</b>	FC, IHC, WB
<b>Application Note:</b>	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.
<b>Assay Dilutions:</b>	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
<b>ELISA:</b>	1:10,000 - 1:50,000
<b>FC:</b>	1:800
<b>IHC:</b>	1:100
<b>WB:</b>	1:1,000

## Formulation

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

**Stabilizer:** None

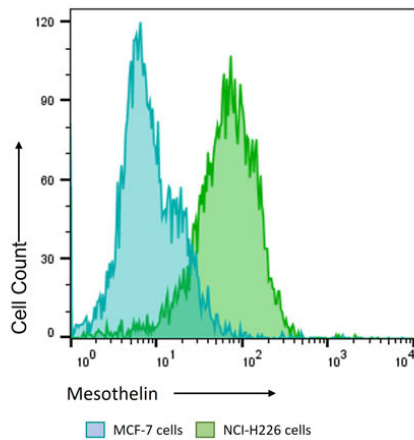
## Shipping & Handling

**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.

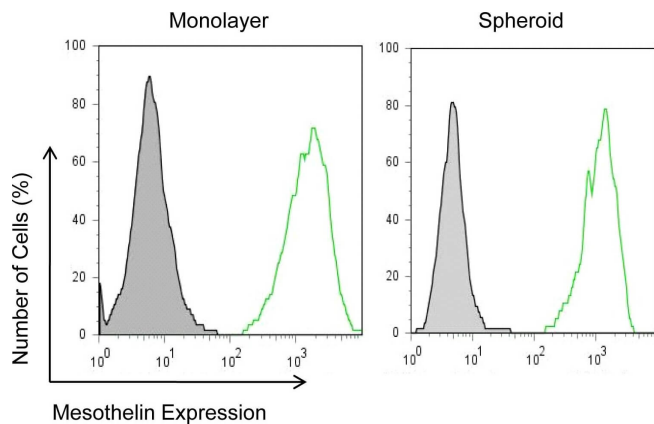
**Expiration:** Expiration date is one (1) year from date of receipt.

## Images



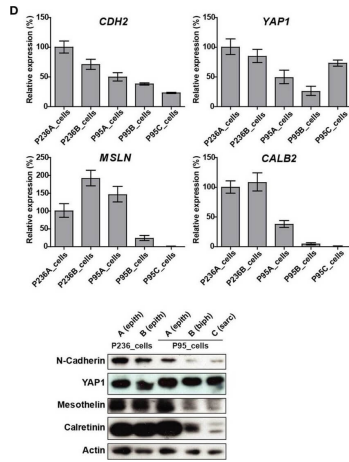
### Flow Cytometry

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.



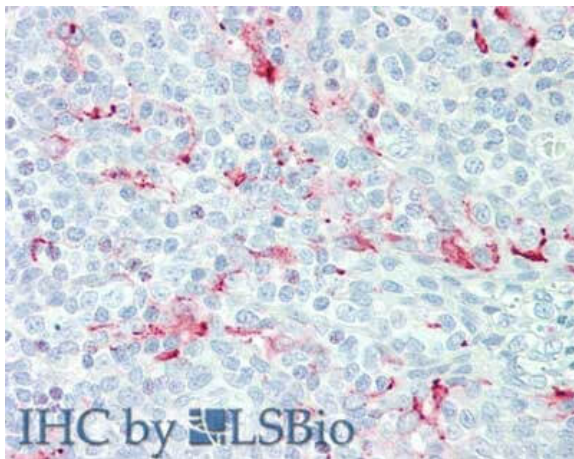
### Flow Cytometry

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.



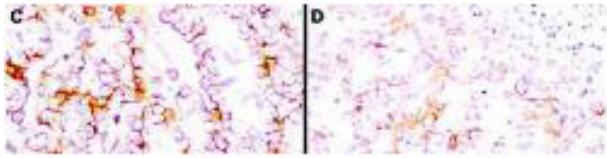
### Western Blot

(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  $\mu$ m. (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.



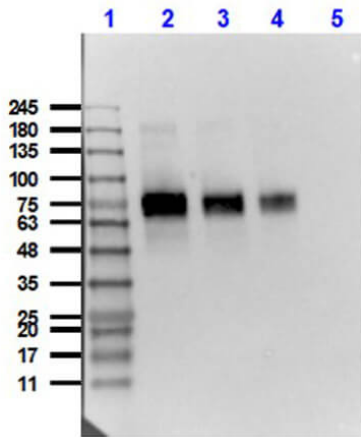
### Immunohistochemistry

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  $\mu$ g/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.



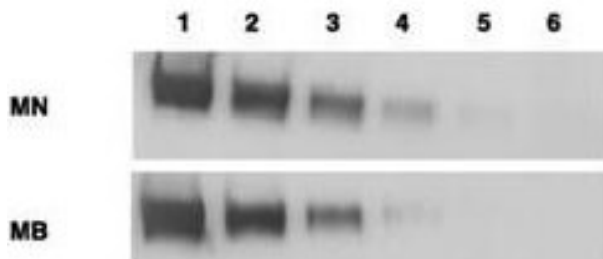
### Immunohistochemistry

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.

## References

- Suurs FV et al. Mesothelin/CD3 half-life extended bispecific T-cell engager molecule shows specific tumor uptake and distributes to mesothelin and CD3 expressing tissues. *J Nucl Med.* (2021)
- Jewell ML et al. Single-Cell RNA Sequencing Identifies Yes-Associated Protein 1–Dependent Hepatic Mesothelial Progenitors in Fibrolamellar Carcinoma. *Am J Pathol.* (2020)
- Inoue S. et al. Diffuse mesothelin expression leads to worse prognosis through enhanced cellular proliferation in colorectal cancer. *Oncology Letters* (2020)
- Oehl et al. Live-Cell Mesothelioma Biobank to Explore Mechanisms of Tumor Progression. *Frontiers in Oncology* (2018)
- Leshem, Y et al. Combining Local Immunotoxins Targeting Mesothelin with CTLA-4 Blockade Synergistically Eradicates Murine Cancer by Promoting Anticancer Immunity. *Cancer Immunology Research* (2017)
- Zhang et al. Efficacy of Anti-mesothelin Immunotoxin RG7787 plus Nab-Paclitaxel against Mesothelioma Patient-Derived Xenografts and Mesothelin as a Biomarker of Tumor Response. *Clinical Cancer Research* (2017)
- Illei, PB et al. Mesothelin Expression in Advanced Gastroesophageal Cancer Represents a Novel Target for Immunotherapy. *Applied Immunohistochemistry & Molecular Morphology : Aimm* (2016)
- Awuah et al. Reduced Shedding of Surface Mesothelin Improves Efficacy of Mesothelin-Targeting Recombinant Immunotoxins. *Molecular Cancer Therapeutics* (2016)
- Khanna et al. Malignant Mesothelioma Effusions Are Infiltrated by CD3+ T Cells Highly Expressing PD-L1 and the PD-L1+ Tumor Cells within These Effusions Are Susceptible to ADCC by the Anti-PD-L1 Antibody Avelumab. *Journal of Thoracic Oncology* (2016)
- Alewine, C et al. Efficacy of RG7787, a next-generation mesothelin-targeted immunotoxin, against triple-negative breast and gastric cancers. *Molecular Cancer Therapeutics* (2014)
- Zhang, J et al. Megakaryocytic potentiating factor and mature mesothelin stimulate the growth of a lung cancer cell line in the peritoneal cavity of mice. *PLoS One* (2014)
- Zhang, J et al. Loss of mesothelin expression by mesothelioma cells grown in vitro determines sensitivity to anti-mesothelin immunotoxin SS1P. *Anticancer Research* (2012)
- Xiang X et al. The development and characterization of a human mesothelioma in vitro 3D model to investigate immunotoxin therapy. *PLoS One.* (2011)
- Feng, M et al. In vivo imaging of human malignant mesothelioma grown orthotopically in the peritoneal cavity of nude mice. *Journal of Cancer* (2011)

## Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.