

**Datasheet for 200-301-A87S****Mesothelin Antibody****Overview**

|                      |                                                            |
|----------------------|------------------------------------------------------------|
| <b>Description:</b>  | Anti-Mesothelin (MOUSE) Monoclonal Antibody - 200-301-A87S |
| <b>Item No.:</b>     | 200-301-A87S                                               |
| <b>Size:</b>         | 25 µL                                                      |
| <b>Applications:</b> | FC, IHC, WB                                                |
| <b>Reactivity:</b>   | Human                                                      |
| <b>Host Species:</b> | Mouse                                                      |

**Product Details**

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|----------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Background:</b>   | Mesothelin is a glycosyl-phosphatidyl- inositol–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 glycosylphosphatidyl-inositol–anchored protein that composes the mature portion and an NH <sub>2</sub> -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>     | MB-G10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <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 - 53988378</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>                  | 1:200                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <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

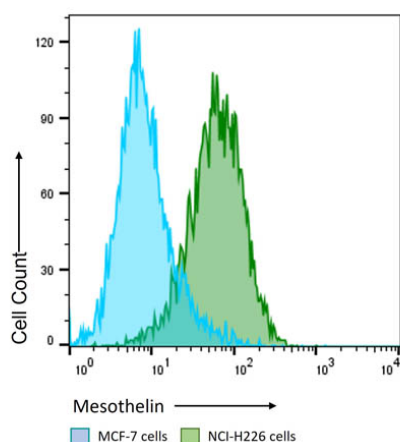
## Shipping & Handling

Shipping Condition: Dry Ice

**Storage Condition:** Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.

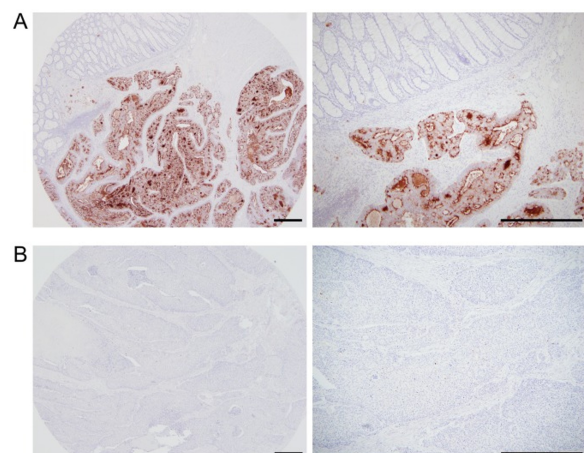
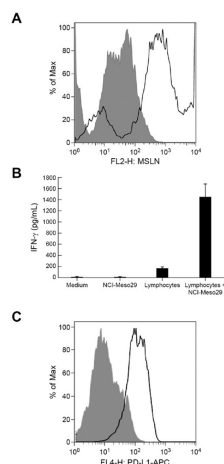
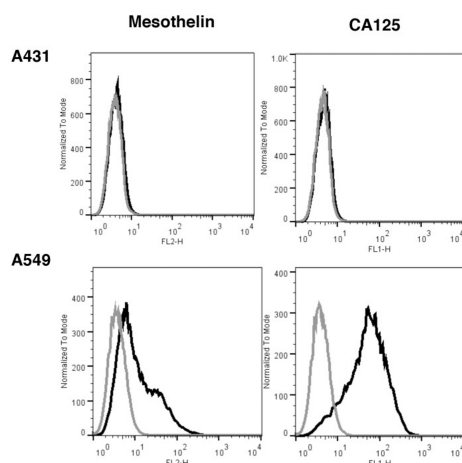
**Expiration:** Expiration date is three (3) months from date of receipt.

## 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 1:200 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

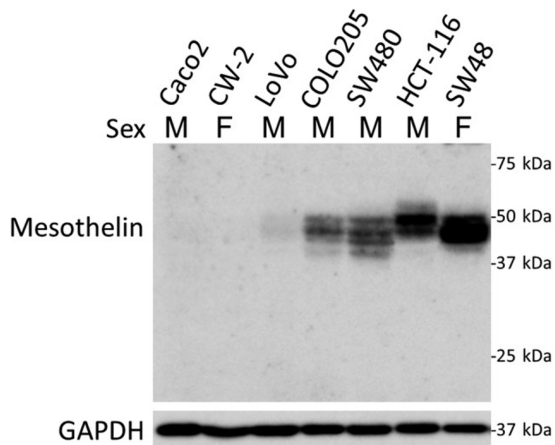
Cells were incubated with the indicated primary antibodies or isotype control antibodies, and appropriate secondary antibody (goat anti-mouse IgG, R-PE or goat anti-mouse, FITC). Results are shown as histogram plots for binding of primary antibody (black trace) or isotype control (gray trace). A431 cells are negative for both MSLN and CA125. A549 cells show low mesothelin expression ( $3.5 \times 10^3$  sites/cell) but highly express CA125. Fig3. PMID: 25118887

### Flow Cytometry

(A) Tumor cells present in ascites were evaluated by their positivity for the tumor differentiation antigen mesothelin by flow cytometry using mouse anti-mesothelin (p/n 200-301-A87) primary antibody followed by goat anti-mouse IgG PE labeled secondary antibody. Shaded histogram represents the binding of isotype control antibody and solid histograms represent the binding of anti-mesothelin antibody. (B) Reactivity of lymphocytes with autologous tumor cells was evaluated by measuring IFN- $\gamma$  released in the supernatants of co-cultures of lymphocytes and tumor cells. (C) Flow cytometry analysis of PD-L1 expression on autologous tumor cells obtained from ascites of patient NCI-Meso29 grown in the presence (solid) or absence (shaded) of autologous lymphocytes cultured from ascites and 106 IU/mL IL-2. Fig 4. PMID: 27544053

### Immunohistochemistry

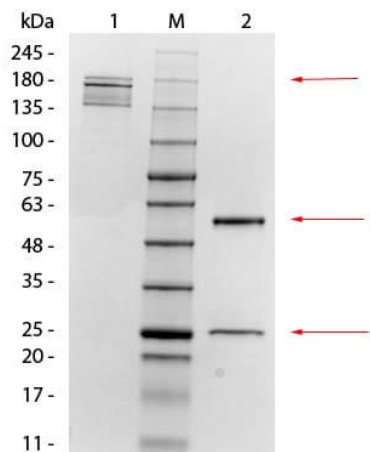
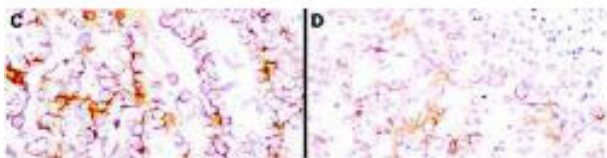
MSLN immunohistochemistry in colon cancer tissue. (A) Case of tubular adenocarcinoma. Diffuse and luminal MSLN expression was detected. (B) Case of poorly differentiated adenocarcinoma exhibiting solid/sheet-like proliferation with undetectable MSLN expression. Left, low-power magnification; right, high-power magnification. Scale bar, 500 μm. MSLN, mesothelin. Fig 1. PMID: 32194667

**Western Blot**

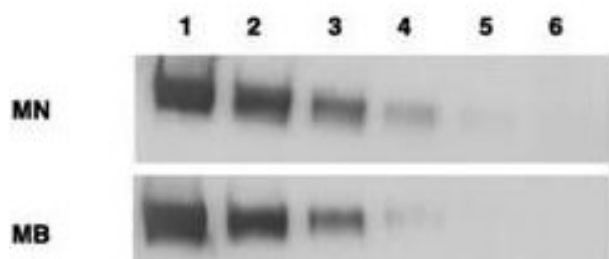
Expression of MSLN in cultured colon cancer cells. MSLN was expressed in four of seven colon cancer cell lines with no association with sex. MSLN, mesothelin; F, female; M, male. Fig 3. PMID: 32194667

**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.

**SDS-PAGE**

SDS-PAGE of Mouse anti-Mesothelin Monoclonal Antibody. Lane 1: Non-Reduced Mouse anti-Mesothelin Monoclonal Antibody. Lane M: 3 µL OPAL Pre-stained Marker (p/n MB-210-0500). Lane 2: Reduced Mouse anti-Mesothelin Monoclonal Antibody. Load: 1 µg per lane. Predicted/Observed size: Non-reduced at 160 kDa; Reduced at 55, 25 kDa.



#### Western Blot

Western blotting using Rockland's anti-mesothelin antibody.

Load: Mesothelin-Fc (lane 1, 100 ng; lane 2, 25 ng; lane 3, 6 ng; lane 4, 2 ng; and lane 5, 0.4 ng) and CD25-Fc (lane 6, 50 ng) Primary antibody: anti-mesothelin at 1mg/ml.

Secondary Antibody: ALP goat anti-mouse IgG and BCIP/NBT substrate. Reprinted with permission from Clin.Cancer Res. 11(16):5840-6.

## References

- Koukoulis K, et al. Targeting Mesothelin via the native T cell receptor. *BioRxiv (Preprint)* (2025)
- Marin-Muller C et al. Nanoparticle-Mediated Therapy with miR-198 Sensitizes Pancreatic Cancer to Gemcitabine Treatment through Downregulation of VCP-Mediated Autophagy. *Pharmaceutics*. (2023)
- Inoue S. et al. Diffuse mesothelin expression leads to worse prognosis through enhanced cellular proliferation in colorectal cancer. *Oncology Letters* (2020)
- 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)
- 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)

## Disclaimer

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