OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26513AF-N

PDPN Rat Monoclonal Antibody [Clone ID: 8F11]

Product data:

Product Type:	Primary Antibodies
Clone Name:	8F11
Applications:	FC, IHC, IP, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. For details see protocols below. **Immunoprecipitation:** 1-2 µg/250 µl of cell extract from 2.5x10^6 cells. **Immunohistochemistry on Paraffin Sections:** 1.25 µg/ml (whole mount). **Flow Cytometry:** 5-10 µg/ml (final concentration). **Immunocytochemistry**: It is reported that this antibody can be used in immunocytochemistry (see reference no. 2)
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	NL-17 cell membrane fraction
Specificity:	This antibody reacts with Mouse Aggrus (Podoplanin). The reference Kato, Y., et al. (2003) described that this antibody does not react to Human Aggrus (Podoplanin).
Formulation:	PBS containing 50% glycerol, pH 7.2 State: Azide Free State: Liquid Ig fraction Preservative: None
Concentration:	lot specific
Purification:	Protein G agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliqouts) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.

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Predicted Protein Size:	44 kDa
Gene Name:	podoplanin
Database Link:	Q86YL7
Background:	In addition to hemostasis and host defense, platelets are involved in the induction of inflammation, tissue repair, and tumor metastasis. Aggrus (T1a/Podoplanin) is a novel 36-45 kDa membrane sialoglycoprotein that induces platelet aggregation. Aggrus is highly expressed in various tumor cells and the platelet aggregation ability of Aggrus depends on specific oligosaccharide structures that may be related to the metastasis process in cancer cells. Aggrus has been identified as a diagnostic tumor marker for seminomas and testicular cancers.
Synonyms:	Glycoprotein 36, PA2.26 antigen, T1-alpha, Aggrus, PDPN, GP36, PSEC0003, PSEC0025

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Note: This product was originally produced by MBL International.

Protocol:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 anti-rat IgG-HRP diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.
(Positive controls for Western blotting; transfectant, NL-17)

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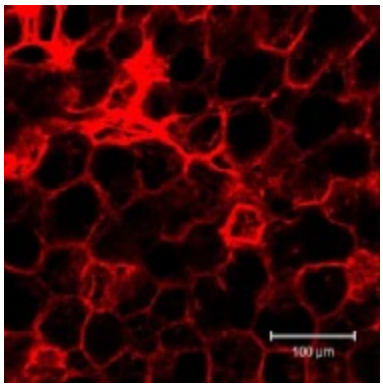
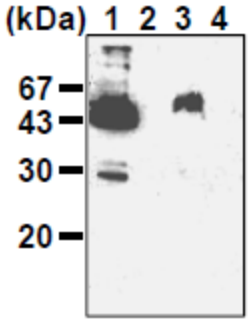
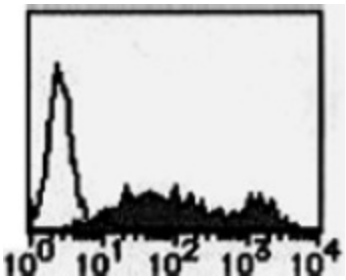
Product images:



Immunohistochemical detection (whole mount) of mouse Aggrus (Podoplanin) on paraffin embedded section of mouse normal lung with AM26513AF-N.



Western blot analysis of mouse Aggrus (Podoplanin)
Lane 1: mouse Aggrus (Podoplanin)/CHO
Lane 2: parental cell (CHO)
Lane 3: NL-17
Lane 4: NL-14
Immunoblotted with AM26513AF-N



Flow cytometric analysis of mouse Aggrus (Podoplanin)/CHO
Closed: AM26513AF-N
Open: Isotype control

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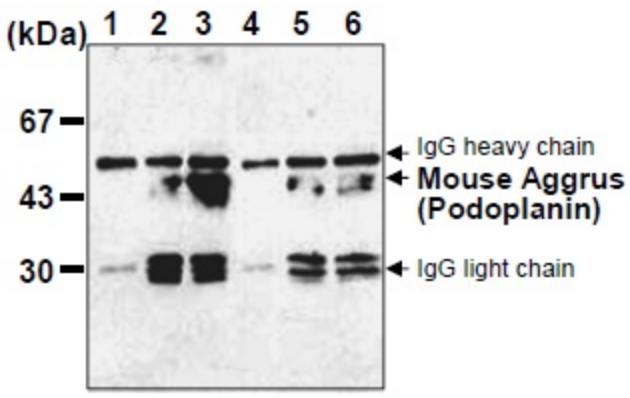

Immunoprecipitation of mouse Aggrus (Podoplanin)
Cell
Lane 1-3:NL-17
Lane 4-6:NL-14
Immunoprecipitation
Lane 1 and4: IP with isotype control,1µg
Lane 2 and 5: IP with AM26513AF-N, 1µg
Lane 3 and 6: IP with AM26513AF-N, 2µg
Immunoblotted with AM26513AF-N

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