Technical Data Sheet

Purified Rat Anti-Human Podoplanin (PA Tag)

Product Information

Material Number:	**572180**
Alternate Name:	AGGRUS; GP36; GP40; Gp38; PA2.26 antigen; PDPN; T1A
Entrez Gene ID:	10630
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	NZ-1
Immunogen:	Human Podoplanin Peptide (aa 38-51)
Isotype:	Rat IgG2a, λ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The NZ-1 monoclonal antibody specifically binds with high affinity to Podoplanin. This antibody was generated by immunizing mice with a 14-residue peptide segment (aa 38-51) from a platelet aggregation-stimulating (PLAG) domain of human Podoplanin. For this reason, this antibody is useful to detect the Podoplanin molecule as well as target proteins, such as antibodies that are labeled with a dodecapeptide (GVAMPGAEDDVV) named PA Tag. Podoplanin is a ~43 kDa mucin-type 1 transmembrane glycoprotein composed of a serine- and threonine-rich intracellular domain, a single transmembrane domain and a short intracellular domain. This multifunctional glycoprotein is expressed on various cell types, including intestinal and thymic epithelial cells, lymphatic endothelial cells, kidney podocytes, alveolar type I cells, fibroblastic reticular cells, macrophages, Th17 cells and osteocytes. Podoplanin expression is upregulated in inflammation and cancer. The binding of Podoplanin to the C-type lectin domain family 1 member B (Clec1B, also known as CLEC-2) on the surface of platelets results in platelet aggregation/activation, thrombosis, lymphatic vessel development, and cancer invasion and metastasis. The Podoplanin (PA Tag) antibody can be used for detecting tagged target proteins in cells that do not express endogenous Podoplanin. This antibody specifically reacts with the human Podoplanin sequence and does not react with Podoplanin sequences from mouse, rat, hamster or dog. Reportedly, this antibody can be used for Western blot, immunohistochemistry, immunoprecipitation or imaging applications.

Tissue culture supernatant is purified by either protein A/G or affinity purification methods. Both methods yield antibody in solution that is free of most other soluble proteins, lipids, etc. This format provides pure antibody that is suitable for a number of downstream applications including: secondary labeling for flow cytometry or microscopy, ELISA, Western blot, etc.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C.

Application Notes

Application

Flow cytometry	Routinely Tested
Intracellular staining (flow cytometry)	Tested During Development
Western blot	Reported
Immunoprecipitation	Reported
Immunohistochemistry	Reported

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
553996	Purified Rat IgG2a, λ Isotype Control	0.5 mg	B39-4
550767	PE Goat Anti-Rat Ig	0.2 mg	Polyclonal
554655	Fixation Buffer	100 mL	(none)
554723	Perm/Wash Buffer	100 mL	(none)
564907	DAPI Solution	1 mg	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.

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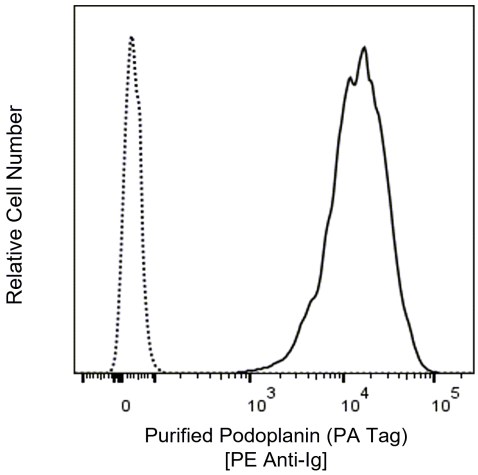
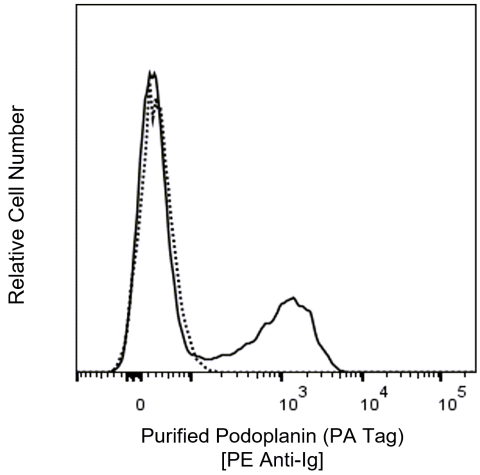
2. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
3. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
4. An isotype control should be used at the same concentration as the antibody of interest.
5. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
6. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
7. For U.S. patents that may apply, see bd.com/patents.



Flow cytometric analysis of Podoplanin (PA Tag) expression on viable NCCIT cells. *Cells from the Human NCCIT (Pluripotent embryonal carcinoma, ATCC® CRL-2073™) cell line were stained with either BD Pharmingen™ Purified Rat IgG2a, λ Isotype Control (Cat. No. 553996; dashed lined histogram) or BD Pharmingen™ Purified Rat Anti-Human Podoplanin (PA Tag) antibody (Cat. No. 572180; solid line histogram) at 0.25 µg/test. The cells were then secondarily stained with BD Pharmingen™ PE Goat Anti-Rat Ig antibody (Cat. No. 550767). DAPI Solution (Cat. No. 564907) was added to cells right before analysis. The fluorescence histogram showing Podoplanin (PA Tag) expression (or Ig Isotype control staining) was derived from gated events with the forward and side light-scatter characteristics of viable (DAPI-negative) cells. Flow cytometry and data analysis were performed using a BD LSRFortessa™ X-20 Cell Analyzer System and FlowJo™ Software. Data shown on this Technical Data Sheet are not lot specific.*



Flow cytometric analysis of PA Tag expression in transfected cells. *Transfected cells with an intracellular protein linked with PA were fixed with BD Cytofix™ Fixation Buffer (Cat. No. 554655) and permeabilized with Perm/Wash Buffer (Cat. No. 554723). The cells were then stained with either BD Pharmingen™ Purified Rat IgG2a, λ Isotype Control (Cat. No. 553996; dashed lined histogram) or BD Pharmingen™ Purified Rat Anti-Human Podoplanin (PA Tag) antibody (Cat. No. 572180; solid line histogram) at 0.25 µg/test. The cells were then secondarily stained with BD Pharmingen™ PE Goat Anti-Rat Ig antibody (Cat. No. 550767). The fluorescence histogram showing PA Tag expression (or Ig isotype staining) was derived from gated events with the forward and side light-scatter characteristics of intact cells. Flow cytometry and data analysis was performed using a BD LSRFortessa™ X-20 Cell Analyzer System and FlowJo™ Software. Data shown on this Technical Data Sheet are not lot specific.*

References

Brown ZP, Takagi J. The PA Tag: A Versatile Peptide Tagging System in the Era of Integrative Structural Biology . *Adv Exp Med Biol.* 2018; 1105:59-76. (Clone-specific: Western blot)

Fujii Y, Kaneko M, Neyazaki M, Nogi T, Kato Y, Takagi J. PA tag: a versatile protein tagging system using a super high affinity antibody against a dodecapeptide derived from human podoplanin. *Protein Expr Purif.* 2014; 95:240-7. (Clone-specific: Flow cytometry, Western blot)

Fujii Y, Matsunaga Y, Arimori T, et al. Tailored placement of a turn-forming PA tag into the structured domain of a protein to probe its conformational state . *J Cell Sci.* 2016; 129(7):1512-22. (Clone-specific: Immunoprecipitation)

Kato Y, Kaneko MK, Kuno A, et al. Inhibition of tumor cell-induced platelet aggregation using a novel anti-podoplanin antibody reacting with its platelet-aggregation-stimulating domain. *Biochem Biophys Res Commun.* 2006; 349(4):1301-7. (Immunogen: Flow cytometry, Immunohistochemistry, Western blot)

Tamura-Sakaguchi R, Aruga R, Hirose M, et al. Moving toward generalizable NZ-1 labeling for 3D structure determination with optimized epitope-tag insertion. *Acta Crystallogr D Struct Biol.* 2021; 77(Pt 5):645-662. (Clone-specific: Immunoprecipitation)