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Not for use in diagnostic procedures.

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Anti-*P. acnes* mAb (PAB antibody)

CODE No. D371-3

CLONALITY Monoclonal
CLONE TMDU2
ISOTYPE Mouse IgM κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgM from mouse ascites fluid
IMMUNOGEN Whole bacterial lysate of *P. acnes* (ATCC® 11828™)
FORMULATION PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

Western blotting 1 µg/mL
Immunohistochemistry 0.25 µg/mL (paraffin section)
Heat treatment for paraffin embedded section: microwave oven, for 40 min. at 97°C in 10 mM citrate buffer (pH 6.2)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Other
Sample	Not tested	Not tested	Not tested	*P. acnes*
Reactivity				+

Note that lipofuscin pigments cause a non-specific staining of PAB antibody.

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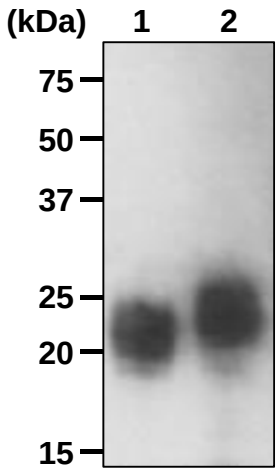
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Suspend the sample with PBS and sonicate 3 times for 5 min.
2) Mix the sample with equal volume of Laemmli's sample buffer.
3) Boil the sample for 3 min. and centrifuge. Load 1 µL (50 µg) of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% acrylamide) for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% Methanol). See the manufacturer's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
8) Incubate the membrane with 1:5,000 of Anti-IgG (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3).
10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; *P. acnes*)



Western blotting analysis of P. acnes
Lane 1: Serotype I *P. acnes* (ATCC® 6919™)
Lane 2: Serotype II *P. acnes* (ATCC® 11828™)

Immunoblotted with Anti-*P. acnes* mAb (PAB antibody) (D371-3)

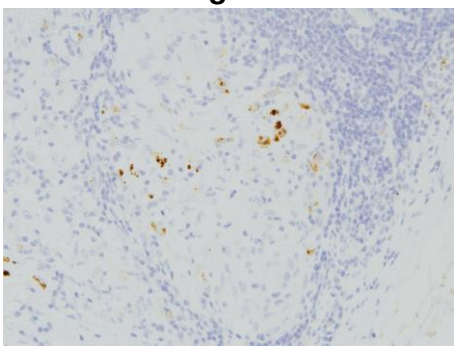
Immunohistochemistry for formalin fixed paraffin-embedded section

Protocol for manual method

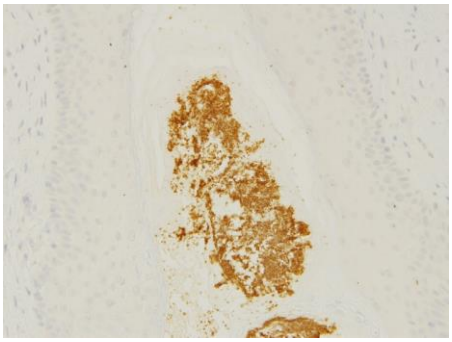
1) Deparaffinize tissue sections in Xylene 3 times for 3 min. each.
2) Immerse the slides with Ethanol 3 times for 3 min. each, then wash the slides in PBS 3 times for 3 min. each.
3) Remove the slides from PBS and heat-treat with 10 mM Citrate buffer (pH 6.2) for 40 min. at 97°C using microwave oven.
4) Let the slide cool down until at room temperature in the Citrate buffer.
5) Remove the slides from the Citrate buffer and inactivate endogenous peroxidase with 3% H_2O_2 in Methanol for 10 min.
6) Wash the slides with PBST [0.25% Tween-20 in PBS] 3 times for 5 min. each.
7) Incubate the sections with 2.5% normal horse serum (Vectastain Universal Elite ABC Kit, Vector Laboratories, code no. PK-7200) for 30 min. at room temperature to block non-specific staining. Do not wash.
8) Incubate the sections with primary antibody diluted with DAKO REAL Antibody diluent (Dako, code no. S2022) as suggested in the **APPLICATIONS** overnight at room temperature (The concentration of antibody will depend on the conditions.).
9) Wash the slides 3 times in PBST for 5 min. each.
10) Incubate the sections with Biotinylated anti-mouse/rabbit IgG (Vectastain Universal Elite ABC Kit) for 30 min. at room temperature.
11) Wash the slides 3 times in PBST for 5 min. each.
12) Incubate the sections with ABC reagent (Vectastain Universal Elite ABC Kit). Incubate for 30 min. at room temperature.
13) Wash the slides 3 times in PBST for 5 min. each.
14) Visualize by reacting for 8 min. with Histofine Simplestain DAB Solution (Nichirei Biosciences, code no. 415171). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
15) Wash the slides twice in PBS for 5 min. each.
16) Counterstain in hematoxylin for 5 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min.
17) Dehydrate by immersing in Ethanol 3 times for 5 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive controls for Immunohistochemistry; Sarcoid granuloma and hair follicle)

Sarcoid granuloma



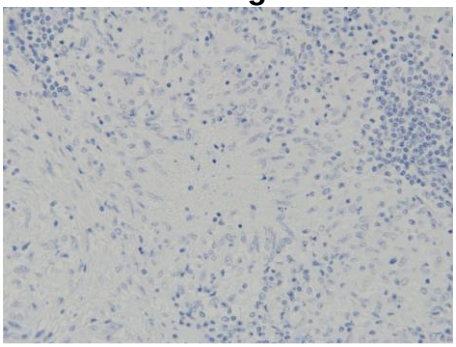
***P. acnes* in hair follicle**



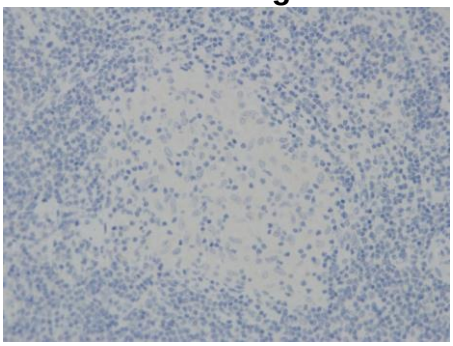
Immunohistochemical detection of P. acnes in human tissue

Brown: Anti-*P. acnes* mAb (PAB antibody) (D371-3)
Blue: Hematoxylin

Tuberculosis granuloma



Sarcoid reaction granuloma



The data were kindly provided by Prof. Yoshinobu Eishi[1] and Mr. Keisuke Uchida[2].
([1]Department of Food and Nutrition, Japan Women's University, [2]Division of Surgical Pathology, Tokyo Medical and Dental University Hospital).

<Appendix>

The following information is the conditions when using automated slide-staining systems. This information was kindly provided by Dr. Yoshinobu Eishi[1] and Mr. Keisuke Uchida[2]. (1: Department of Food and Nutrition, Japan Women's University, 2: Division of Surgical Pathology, Tokyo Medical and Dental University Hospital)
The following methods are introduced in the order that showed high sensitivity results in their study.

Note: Please establish appropriate condition by each user referring to this information.
Please ask each manufacturer for the details of the instruments and reagents.

A) Protocol for Leica BOND III

Working dilution; 1:500 – 1:4000
Detection system: BOND Polymer Refine Detection (Leica, code no. DS9800)
Immunostaining procedure;

Dewax					
	Step regent	**Step type:**	**Incubation time:**	**Temprature:**	**Dispense type:**
1	Bond Dewax Solution	Reagent	30sec	72°C	150µL
2	Bond Dewax Solution	Reagent	0sec	72°C	150µL
3	Bond Dewax Solution	Reagent	0sec	72°C	150µL
4	Alcohol	Wash	0sec	Ambient	150µL
5	Alcohol	Wash	0sec	Ambient	150µL
6	Alcohol	Wash	0sec	Ambient	150µL
7	Bond Wash Solution	Wash	0sec	Ambient	150µL
8	Bond Wash Solution	Wash	0sec	Ambient	150µL
9	Bond Wash Solution	Wash	0sec	Ambient	150µL
Pretreatment					
	Step regent	**Step type:**	**Incubation time:**	**Temprature:**	**Dispense type:**
1	Bond ER Solution 1	Reagent	0sec	Ambient	150µL
2	Bond ER Solution 1	Reagent	0sec	Ambient	150µL
3	Bond ER Solution 1	Reagent	60min	100°C	Intermediate
4	Bond ER Solution 1	Reagent	0sec	Ambient	150µL
5	Bond Wash Solution	Wash	0sec	Ambient	150µL
6	Bond Wash Solution	Wash	0sec	Ambient	150µL
7	Bond Wash Solution	Wash	0sec	Ambient	150µL
8	Bond Wash Solution	Wash	0sec	Ambient	150µL
9	Bond Wash Solution	Wash	3min	Ambient	150µL
IHC staining					
	Step regent	**Step type:**	**Incubation time:**	**Temprature:**	**Dispense type:**
1	Bond Wash Solution	Reagent	0sec	Ambient	150µL
2	Bond Wash Solution	Wash	1min	Ambient	Open
3	Bond Wash Solution	Wash	0sec	Ambient	Open
4	Bond Wash Solution	Wash	1min	Ambient	150µL
5	PAB antibody	Reagent	15min	Ambient	150µL
6	Bond Wash Solution	Reagent	0sec	Ambient	150µL
7	Bond Wash Solution	Wash	1min	Ambient	150µL
8	Bond Wash Solution	Wash	0sec	Ambient	150µL
9	Bond Wash Solution	Wash	1min	Ambient	150µL
10	Bond Wash Solution	Wash	0sec	Ambient	150µL
11	Post Primary	Reagent	8min	Ambient	150µL
12	Bond Wash Solution	Wash	2min	Ambient	150µL
13	Bond Wash Solution	Wash	0sec	Ambient	150µL
14	Bond Wash Solution	Wash	2min	Ambient	150µL
15	Bond Wash Solution	Wash	2min	Ambient	150µL
16	Bond Wash Solution	Wash	2min	Ambient	150µL
17	Polymer	Reagent	8min	Ambient	150µL
18	Bond Wash Solution	Wash	2min	Ambient	150µL
19	Bond Wash Solution	Wash	0sec	Ambient	150µL
20	Bond Wash Solution	Wash	2min	Ambient	150µL
21	Bond Wash Solution	Wash	2min	Ambient	150µL
22	Bond Wash Solution	Wash	2min	Ambient	150µL
23	Peroxide Block	Reagent	5min	Ambient	150µL
24	Bond Wash Solution	Wash	0min	Ambient	150µL
25	Bond Wash Solution	Wash	1min	Ambient	150µL

26	Bond Wash Solution	Wash	0min	Ambient	150µL
27	Deionized Water	Wash	1min	Ambient	150µL
28	Mixed DAB Refine	Reagent	0min	Ambient	150µL
29	Mixed DAB Refine	Reagent	10min	Ambient	150µL
30	Deionized Water	Wash	0min	Ambient	150µL
31	Deionized Water	Wash	1min	Ambient	150µL
32	Deionized Water	Wash	0min	Ambient	150µL
33	Deionized Water	Wash	1min	Ambient	150µL
34	Hematoxylin	Reagent	5min	Ambient	150µL
35	Deionized Water	Wash	0min	Ambient	150µL
36	Bond Wash Solution	Wash	1min	Ambient	150µL
37	Deionized Water	Wash	0min	Ambient	150µL

B) Protocol for DAKO Autostainer (PT LINK and Autostainer Link 48)

Working dilution; 1:500 – 1:4000

Immunostaining procedure;

1. Deparaffinization, dehydration and antigen retrieval treatment performed by PT LINK (DAKO) with Target Retrieval Solution Low pH (DAKO, code no. K8005).
   PAB antibody; Preheat 65°C, 95°C 20 min., cool 65°C (twice)
2. Cool down in PBS or EnVision FLEX WASH BUFFER (15 min.).
3. Set slide glasses on the Autostainer Link 48 (DAKO).

Category	Code	Name	Volume	Incubation
Rinse		Buffer		0
Endogenous Enzyme Block	SM801	FLEX Peroxidase Block	100	5
Rinse		Buffer		0
Primary Antibody		PAB antibody	100	30
Rinse		Buffer		0
Secondary Reagent	SM804	FLEX + Mouse (LINKER)		30
Rinse		Buffer		0
Labelled Polymer	SM802	FLEX / HRP	100	20
Rinse		Buffer		0
Rinse		Buffer		5
Substrate-Chromogen	SM803	FLEX DAB+Sub-Chromo	150	5
Rinse		Buffer		0
Counterstain	SM806	FLEX Hematoxylin	150	5
Rinse		DI Water		0
Rinse		Buffer		5
Rinse		DI Water		0
		(End of Protocol)		

4. Clear and mount through graded alcohol series and xylenes.

C) Protocol for Ventana BenchMark

Working dilution of PAB antibody; 1:500 – 1:4000

Detection system: OptiView

Important notes;

1. Since mineral oil (LS) used the machine seems to inhibit PAB antibody reaction, please wash with EZ buffer and tap water before antibody reaction.
2. For dilution of PAB antibody (1:500 – 1:4000), please use diluent of DAKO S2022 (or PBS). The reaction of PAB antibody is inhibited when Roche diluent or RB buffer is used.
3. To prevent drying of the slide glasses, set the antibody reaction to room temperature.

Immunostaining procedure;

1 Deparaffinization [Selected]
2 Cell Conditioning [Selected]
3 CC1 [Selected]

4	CC1 8 Min [Selected]
5	CC1 16 Min [Selected]
6	CC1 24 Min [Selected]
7	CC1 32 Min [Selected]
8	CC1 40 Min [Selected]
9	CC1 48 Min [Selected]
10	CC1 56 Min [Selected]
11	CC1 64 Min [Selected]
12	Pre Primary Peroxidase Inhibit. [Selected]
13	Primary Antibody [Selected]
14	Primary Antibody Temperature [Selected]
15	Primary Antibody No Heat [Selected]
16	Antibody Titration [Selected]
Outside the machine	**Take the slide glasses, put into the EZ buffer.** **Remove the EZ buffer after 5 min and put the EZ buffer again.** **Remove the EZ buffer after 5 min and put the EZ buffer again.** **Remove the EZ buffer after 5 min and put the EZ buffer again.** **Wash with tap water for 10 min.** **Dip in RB buffer.** **Return the slide glasses to the machine.** **Add PAB antibody (300µl or more) on the slide glasses.**
17	***** ~~Hand Apply (Primary Antibody)~~, and Incubate for [0 Hr 30 Min] *****
18	OptiView HQ Linker [Selected]
19	OptiView HQ Universal Linker [Selected]
20	Apply One Drop of OV HQ UNIV LINKR, Apply Coverslip, and Incubate for [8 Minutes]
21	OptiView HRP Multimer [Selected]
22	Counterstain [Selected]
23	Apply One Drop of [HEMATOXYLIN II] (Counterstain), Apply Coverslip, and Incubate for [8 Minutes]
24	Post Counterstain [Selected]
25	Apply One Drop of [BLUING REAGENT] (Post Counterstain), Apply Coverslip, and Incubate for [4 Minutes]

* one drop is one reagent dispense
Ventana Medical Systems, Inc., 1910 Innovation Park Drive Tucson, Arizona USA
NexES v10.6