

Anti-*T. forsythia* mAb (TF antibody)

CODE No.	D377-3
CLONALITY	Monoclonal
CLONE	CH1-9
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified from hybridoma supernatant using protein A agarose
IMMUNOGEN	Whole bacterial lysate of <i>T. forsythia</i> (ATCC [®] 43037 [™])
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

<u>Western blotting</u>	0.5-1 μ g/mL
<u>Immunohistochemistry</u>	1 μ g/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	<i>T. forsythia</i>
Reactivity				+

REFERENCE 1) Rajakaruna, G. A., *et al.*, *Scientific Reports* **8**, 9507-9519 (2018)

RELATED PRODUCTS

- D377-3 Anti-*T. forsythia* mAb (TF antibody) (CH1-9)
- D376-3 Anti-*P. gingivalis* mAb (PG antibody) (AW1-4)
- D372-3 Anti-Mycobacteria mAb (LAM antibody) (TMDU3)
- D371-3 Anti-*P. acnes* mAb (PAB antibody) (TMDU2)
- D369-3 Anti-*H.pylori* mAb (TMDU-D8)

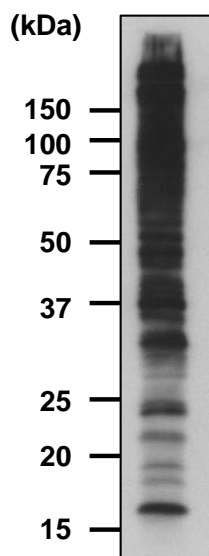
- M075-3 Mouse IgG1 (isotype control) (2E12)

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SDS-PAGE & Western blotting

- 1) Mix the sample with equal volume of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
- 2) Boil the sample for 3 min. and centrifuge. Load 10 μ L (1 μ g) of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² 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.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 5) 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.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 7) Incubate the membrane with 1:10,000 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.
- 8) Wash the membrane with PBS-T (5 min. x 3 times).
- 9) 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.
- 10) Expose to an X-ray film in a dark room for 5 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; *T. forsythia*)



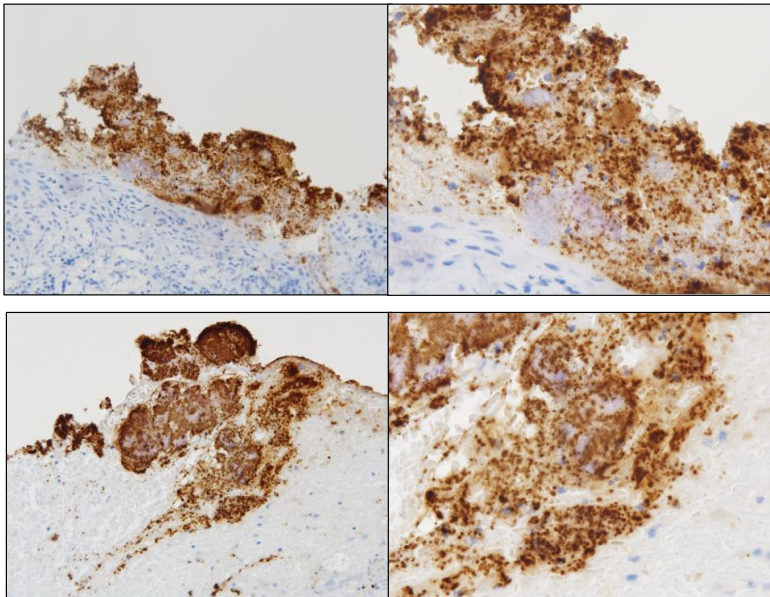
Western blotting analysis of T. forsythia

Immunoblotted with Anti-*T. forsythia* mAb (TF antibody) (D377-3)

Immunohistochemistry for formalin fixed paraffin-embedded section

- 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% H₂O₂ 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 2 times 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 control for Immunohistochemistry; Gingiva tissue)



Immunohistochemical detection of T. forsythia in gingiva tissues.

Brown: Anti-*T. forsythia* mAb (TF antibody) (D377-3)

Blue: Hematoxylin

The data were kindly provided by Prof. Yoshinobu Eishi¹ and Mr. Keisuke Uchida².

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