

**For Research Use Only.**  
**Not for use in diagnostic procedures.**



# Anti-Pfetin (Human) mAb

**CODE No.** D348-3

**CLONALITY** Monoclonal  
**CLONE** 10-4  
**ISOTYPE** Mouse IgG2b  $\kappa$   
**QUANTITY** 100  $\mu$ L, 1 mg/mL

**SOURCE** Purified IgG from hybridoma supernatant  
**IMMUNOGEN** Human Pfetin (recombinant)  
**FORMURATION** 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  $\mu$ g/mL for chemiluminescence detection system  
Immunohistochemistry 1  $\mu$ g/mL (paraffin section)  
Heat treatment for paraffin embedded section: autoclave, for 10 min. in 10 mM citrate buffer (pH 6.0)

## SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	Recombinant protein	Not tested	Not tested	Not tested
Reactivity	+			

**Entrez Gene ID** 115207 (Human)

**REFERENCES**

- 1) Kubota, D., *et al.*, *Jpn. J. Clin. Oncol.* **42**, 730-741 (2012) [IHC-P]
- 2) Kubota, D., *et al.*, *Jpn. J. Clin. Oncol.* **41**, 1194-1202 (2011) [IHC-P]
- 3) Kikuta, K., *et al.*, *Jpn. J. Clin. Oncol.* **40**, 60-72 (2010) [IHC-P]
- 4) Kondo, T., *et al.*, *Proteomics Clin Appl.* **7**, 70-78 (2013)

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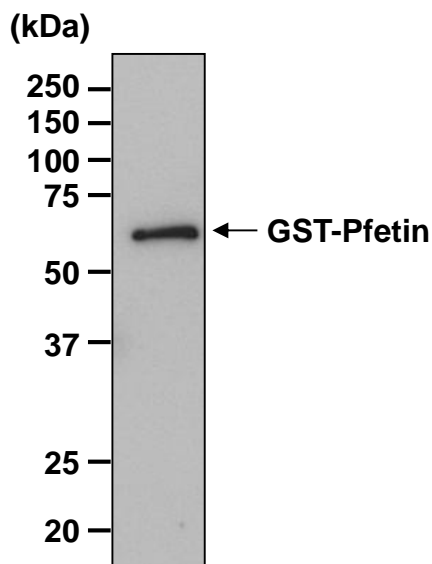
## **RELATED PRODUCTS**

D348-3	Anti-Pfetin (Human) mAb
K0106-3	Anti-CD117 (c-Kit) (Human) mAb
K0105-4	Anti-CD117 (c-Kit) (Human) mAb-FITC
K0106-4	Anti-CD117 (c-Kit) (Human) mAb-FITC
566	Anti-CD117 (c-Kit) (Human) pAb
566-H	Anti-CD117 (c-Kit) (Human) pAb
K0102-3	Anti- $\beta$ -Catenin mAb
CY-M1036	Anti-S100A10 mAb
CY-P1033	Anti-S100A10 pAb
CY-M1037	Anti-S100A11 mAb
CY-P1034	Anti-S100A16 pAb
CY-P1040	Anti-S100A2 pAb
CY-M1039	Anti-S100A3 (Human) mAb
CY-P1039	Anti-S100A3 pAb
CY-P1026	Anti-S100A4/p9Ka pAb
CY-P1028	Anti-S100P pAb
CY-8095	CircuLex S100A10 ELISA Kit
CY-8063	CircuLex S100A11 ELISA Kit
CY-8058	CircuLex S100A12/EN-RAGE ELISA Kit
CY-8057	CircuLex S100A13 ELISA Kit
CY-8064	CircuLex S100A14 ELISA Kit
CY-8086	CircuLex S100A4 ELISA Kit Ver.2
CY-8097	CircuLex S100A6 ELISA Kit
CY-8073	CircuLex S100A7/Psoriasis ELISA Kit
CY-8061	CircuLex S100A8/MRP8 ELISA Kit
CY-8062	CircuLex S100A9/MRP14 ELISA Kit
CY-8060	CircuLex S100P ELISA Kit
CY-R2251	Human S100A1
CY-R2451	Human S100A1 Low Endotoxin
CY-R2260	Human S100A10
CY-R2269	Human S100A11
CY-R2461	Human S100A11 Low Endotoxin
CY-R2262-G	Human S100A12 (GST-tag)
CY-R2262-H	Human S100A12 (His-tag)
CY-R2462-G	Human S100A12 Low Endotoxin
CY-R2263	Human S100A13
CY-R2264	Human S100A14
CY-R2464	Human S100A14 Low Endotoxin
CY-R2266	Human S100A16
CY-R2252	Human S100A2
CY-R2253	Human S100A3
CY-R2453	Human S100A3 Low Endotoxin
CY-R2254	Human S100A4
CY-R2454	Human S100A4 Low Endotoxin
CY-R2255	Human S100A5
CY-R2256	Human S100A6
CY-R2257	Human S100A7
CY-R2457	Human S100A7 Low Endotoxin
CY-R2258	Human S100A8
CY-R2458	Human S100A8 Low Endotoxin
CY-R2259-G	Human S100A9 (GST-tag)
CY-R2259-H	Human S100A9 (His-tag)
CY-R2459-G	Human S100A9 Low Endotoxin
CY-R2250	Human S100B
CY-R2267	Human S100P
CY-R2467	Human S100P Low Endotoxin

### **SDS-PAGE & Western blotting**

- 1) Boil the sample for 2 min. and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 2) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 3) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 4) 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.)
- 5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 6) Incubate the membrane with the 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.
- 7) Wash the membrane with PBS-T (5 min. x 3 times).
- 8) 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.
- 9) 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; GST-tagged human Pfetin)



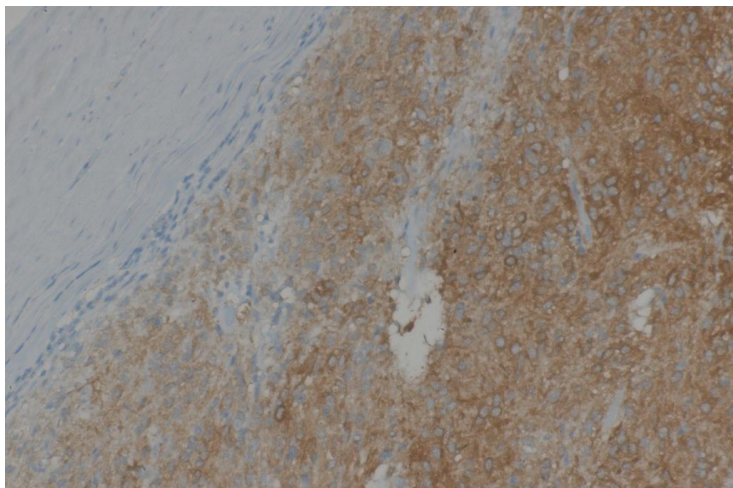
### ***Western blot analysis of Pfetin***

Immunoblotted with Anti-Pfetin (Human) mAb (D348-3)

### **Immunohistochemistry for formalin fixed paraffin-embedded section**

- 1) Deparaffinize tissue sections in Xylene.
- 2) Hydrate the sections through graded Ethanol to water.
- 3) Wash the slides 1 time in distilled water.
- 4) Remove the slides from distilled water and inactivate endogenous peroxidase with 1% H<sub>2</sub>O<sub>2</sub> in Methanol for 30 min.
- 5) Wash the slides with distilled water.
- 6) Remove the slides from distilled water and heat-treated with 10 mM Citrate buffer (pH 6.0) for 10 min. at 121°C using autoclave.
- 7) Let the slide cool down until at room temperature in the Citrate buffer.
- 8) Wash the slides with running water for 5 min., then wash with PBS for 5 min.
- 9) Remove the slides from PBS, and immerse the slides in blocking buffer (10% normal horse serum in PBS) for 5 min. at room temperature to block non-specific staining. Do not wash.
- 10) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with DAKO REAL Antibody diluent (Dako; code no. S2022) as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.) Incubate the sections for 1 hr. at room temperature.
- 11) Wash the slides 2 times in PBS and 1 time in PBST [0.05% Tween-20 in PBS] for 5 min. each.
- 12) Wipe gently around each section and cover tissues with ENVISION (Dako; code no. K4001). Incubate for 30 min. at room temperature.
- 13) Wash the slides 2 times in PBS and 1 time in PBST for 5 min. each.
- 14) Visualize by reacting for 2 min. with DAB Substrate Solution (Dako; code no. K3468). \*DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 15) Wash the slides in distilled water for 5 min.
- 16) Counterstain in hematoxylin for 40 sec., wash the slides in water.
- 17) Dehydrate by immersing in Ethanol, followed by immersing in Xylene. Now ready for mounting.

(Positive control for Immunohistochemistry; GIST primary tumor tissue)



### ***Immunohistochemical detection of Pftin in GIST primary tumor tissue.***

Brown: Anti-Pftin (Human) mAb (D348-3)  
Blue: Hematoxylin

The data was kindly provided by Dr. Tadashi Kondo, M.D., Ph.D.  
(Division of Pharmacoproteomics, National Cancer Center Research Institute)