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

Anti-MELK (Human) mAb

CODE No. D373-3

CLONALITY Monoclonal
CLONE 11-28
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Recombinant protein, corresponding to amino acids 264-601 of human MELK
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 for chemiluminescence detection system
Immunohistochemistry 1-5 µg/mL (paraffin section)
Heat treatment for paraffin embedded section: microwave oven, 2 times for 10 min. in 10 mM citrate buffer (pH 6.0)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Sample	MCF7, ZR-75-1, A549, Caco-2, HEL, KG1, K562, Jurkat, Saos-2, HeLa	Not tested	Not tested	Not tested
Reactivity	+			

Entrez Gene ID 9833 (Human)

REFERENCES

1) Chung, S., *et al.*, *Oncotarget* **7**, 18171-18182 (2016)
2) Chung, S., *et al.*, *Oncotarget* **3**, 1629-1640 (2012)

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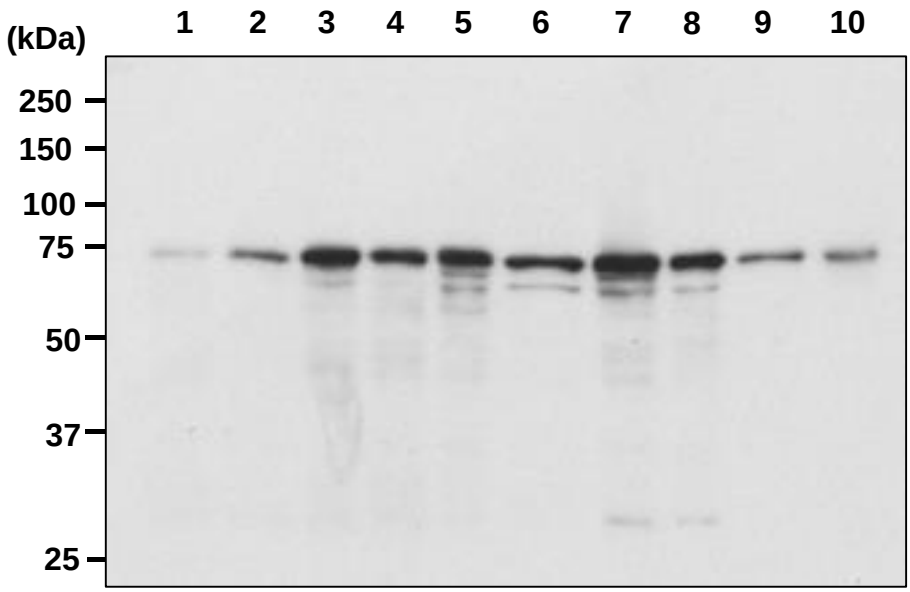
MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
e-mail support@mbl.co.jp, TEL 052-238-1904

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.)
2) Boil the sample for 3 min. and centrifuge. Load 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/cm^2 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.
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 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; MCF7, ZR-75-1, A549, Caco-2, HEL, KG1, K562, Jurkat, Saos-2 and HeLa)



***Western blot analysis of* human MELK**

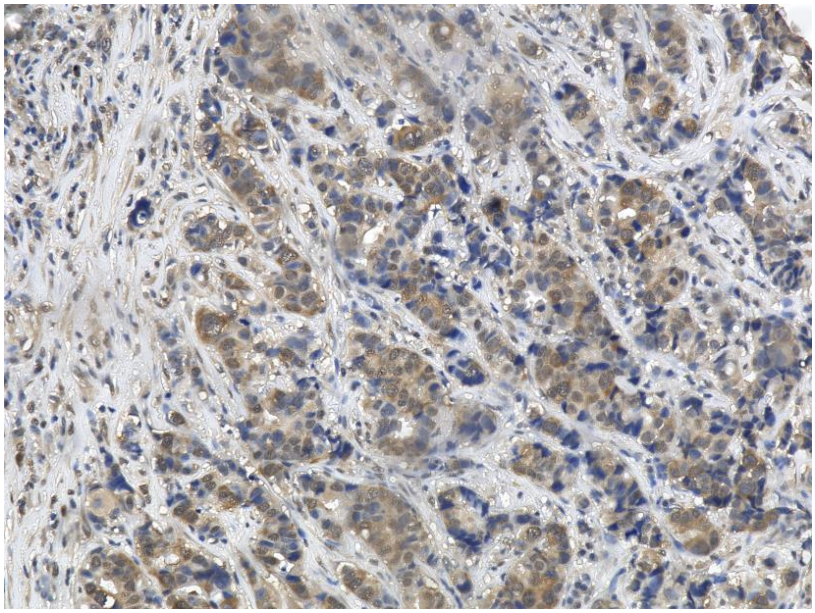
Lane 1: MCF7, 20 μL
Lane 2: ZR-75-1, 20 μL
Lane 3: A549, 10 μL
Lane 4: Caco-2, 20 μL
Lane 5: HEL, 20 μL
Lane 6: KG1, 20 μL
Lane 7: K562, 20 μL
Lane 8: Jurkat, 10 μL
Lane 9: Saos-2, 10 μL
Lane 10: HeLa, 5 μL

Immunoblotted with Anti-MELK (Human) mAb (D373-3)

Immunohistochemistry for formalin fixed paraffin-embedded section

1) Deparaffinize tissue sections in Xylene 3 times for 5 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 6.0) 2 times for 10 min. at 100°C using microwave oven.
4) Let the slide cool down to room temperature within the citrate buffer.
5) Remove the slides from the Citrate buffer and inactivate endogenous peroxidase with 3% H_2O_2 in PBS for 10 min.
6) Wash the slides with PBS 2 times for 5 min. each.
7) Incubate the sections with blocking buffer [20 mM HEPES, 1% BSA, 135 mM NaCl] for 5 min. at room temperature to block non-specific staining. Do not wash.
8) Incubate the sections with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.).
9) Wash the slides 2 times in PBS for 5 min. each.
10) Incubate the section with Histostar (Ms + Rb) (MBL; code no. 8460) for 30 min. at room temperature.
11) Wash the slides 3 times in PBS for 5 min. each.
12) Visualize by reacting for 5 min. with Histostar DAB Substrate Solution (MBL; code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides 2 times in PBS for 5 min. each.
14) 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.
15) Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Human breast cancer tissue)



Immunohistochemical detection of MELK in human breast cancer tissue
Brown: Anti-MELK (Human) mAb (D373-3)
Blue: Hematoxylin