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

MBL
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Anti-Phospho-Keratin-5 (Thr23) mAb

CODE No. D374-3

CLONALITY Monoclonal
CLONE PK5-23
ISOTYPE Rat IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN KLH-conjugated synthetic peptide, CTASAIpTPSVSR (corresponding to amino acids 19-29 of human Keratin-5)
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.

APPLICATION-CONFIRMED

Western blotting	1 µg/mL for chemiluminescence detection system

APPLICATIONS-REPORTED

Immunocytochemistry	Reference 1)
Immunohistochemistry	Reference 1)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse*	Rat	Hamster
Cell	HaCaT in prometaphase or metaphase	Not tested	Not tested	Not tested
Reactivity	+			

*It is reported that clone PK5-23 reacts with mouse tissues in Immunohistochemistry[1)].

Entrez Gene ID 3852 (Human)

REFERENCE

1) Inaba, H., *et al.*, *Biochem. Biophys. Res. Commun.* **498**, 544-550 (2018) [WB, IC, IHC]

For more information, please visit our web site **http://ruo.mbl.co.jp/**

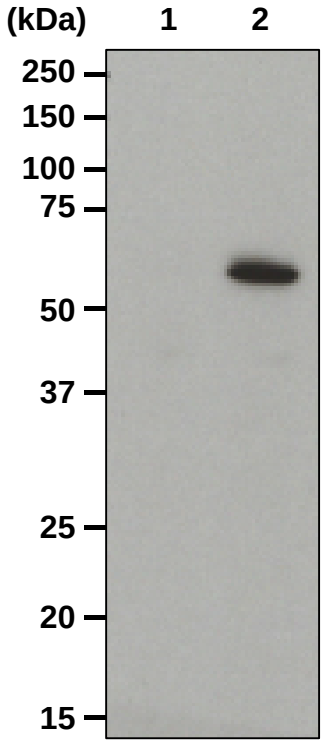
RELATED PRODUCTS

D375-3 Anti-Phospho-Desmin (Ser31) mAb (TD31)
M081-3 Rat IgG2a (isotype control) (2H3)

SDS-PAGE & Western blotting

1) Wash the cells 3 times with PBS and suspend them in 200 μL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.)
2) Boil the sample for 2 min. and centrifuge. Load 5 μL 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/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 **APPLICATION** 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 1:2,000 of Goat anti-Rat IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP (Invitrogen; code no. A10549) 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 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; HaCaT in prometaphase or metaphase)



Western blot analysis of Phospho-Keratin-5 (Thr23) in HaCaT cells

Lane 1: 0 hr. after release from synchronization at the G2/M boundary
Lane 2: 1 hr. after release from synchronization at the G2/M boundary

Immunoblotted with Anti-Phospho-Keratin-5 (Thr23) mAb (D374-3)