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Not for use in diagnostic procedures.

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Anti-LANA (KSHV ORF73) mAb

CODE No.	D325-3
CLONALITY	Monoclonal
CLONE	A23-9
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
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	2 µg/mL
Immunocytochemistry	2 µg/mL

Entrez Gene ID 4961527

REFERENCES

1) Sakakibara, S., *et al.*, *J. Virol.* **78**, 7299-7310 (2004) [WB, IC]

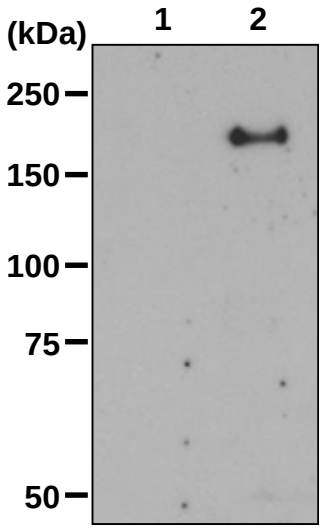
For more information, please visit our web site **https://ruo.mbl.co.jp/.**

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

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (7.5% 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% MeOH). 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) for 1 hr. at room temperature.
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 times).
8) Incubate the membrane with the 1:10,000 anti-IgG (Mouse)-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 times).
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 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; transfectant)

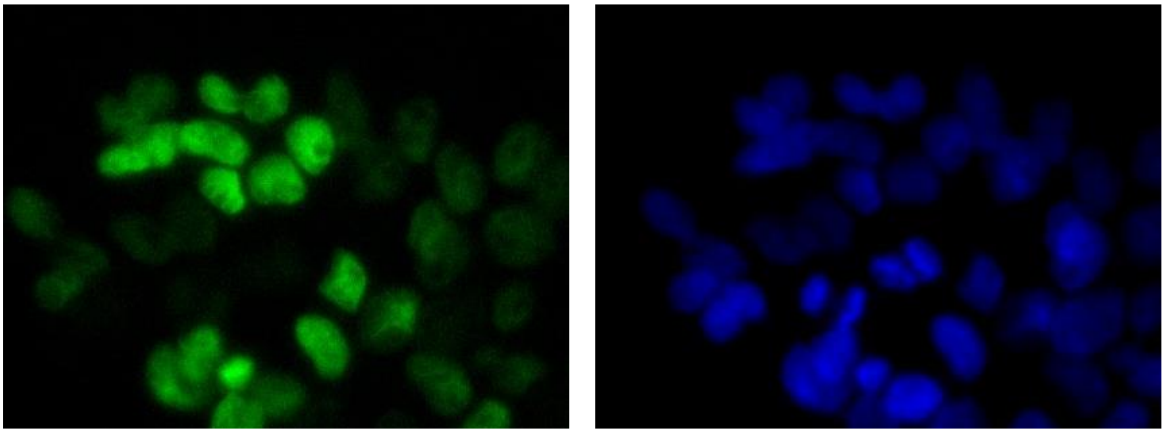


Western blot analysis of LANA
Lane 1: 293
Lane 2: LANA/293
Immunoblotted with D325-3

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25ºC).
4) Wash the slide 2 times in PBS.
5) Immerse the slide in 0.2% Triton X-100/PBS for 10 min. at room temperature.
6) Wash the slide 2 times in PBS.
7) Add Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell and incubate for 5 min. at room temperature.
8) Tip off the blocking buffer, add 200 µL of the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide 2 times in PBS.
10) Add 100 µL of 1:400 anti-IgG (Mouse)-Alexa Fluor®488 (Invitrogen; code no. A11001) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
11) Wash the slide 2 times in PBS.
12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
13) Counter stain with DAPI for 5 min. at room temperature.
14) Wash the slide 1 time in PBS for 5 min.
15) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; transfectant)



Immunocytochemical detection of LANA
Cell: LANA/293

Green: D325-3
Blue: DAPI