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

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Anti-Pseudouridine mAb

CODE No.	D347-3
CLONALITY	Monoclonal
CLONE	APU-6
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH-conjugated pseudouridine
REACTIVITY	This clone reacts with pseudouridine (Ψ) containing RNA. Please see the references for more details.
FORMULATION	PBS containing 50% glycerol. 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

Immunohistochemistry	1 µg/mL (paraffin section)
Immunocytochemistry	1 µg/mL
RNA immunoprecipitation	Not recommended

APPLICATIONS-REPORTED

ELISA	Reference 2) and 3)
Immuno-Northern blotting	Reference 1)

REFERENCES

1) Mishima, E., et al., *PLoS One* **10**, e0143756 (2015) [Immuno-Northern blotting]
2) Masuda, M., *et al*., *Cancer* **72**, 3571-3578 (1993) [IHC-p, ELISA]
3) Itoh, K., *et al., Clin. Chim. Acta* **181** 305-315 (1989) [ELISA]
4) Itoh, K., *et al., Tohoku J. Exp. Med.* **168**, 329-331 (1992)

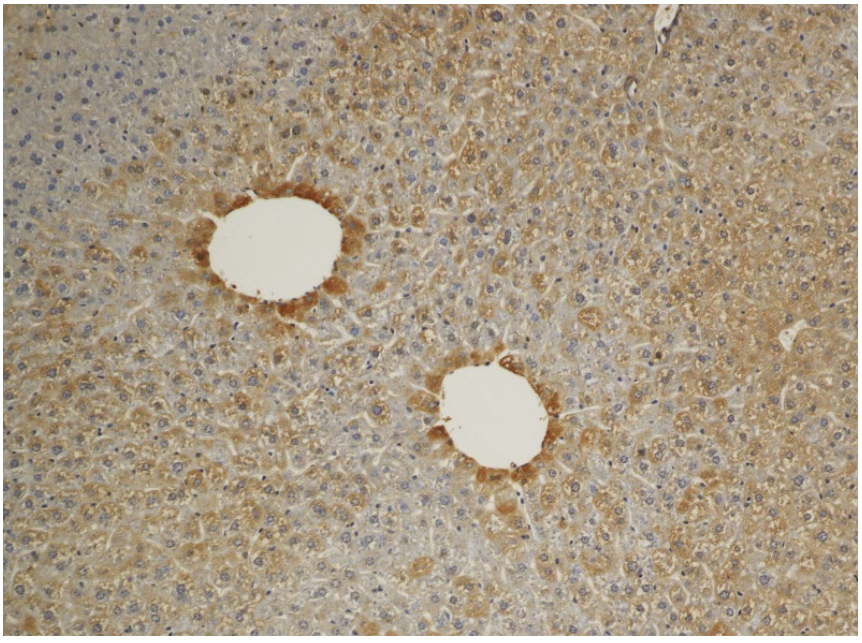
For more information, please visit our web site **https://ruo.mbl.co.jp/**

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

Immunohistochemistry for formalin fixed paraffin-embedded section

1) Deparaffinize the section with Xylene (5 min. x 3).
2) Wash the slide with Ethanol (5 min. x 3).
3) Wash the slide with PBS (5 min. x 3).
4) Remove the slide from PBS and inactivate endogenous peroxidase with 3% H_2O_2 in PBS for 5 min.
5) Wash the slide with PBS (5 min. x 3).
6) Remove the slide from PBS, wipe gently around the section and incubate with blocking buffer [20 mM HEPES/1% BSA/135 mM NaCl] for 10 min. at room temperature to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and incubate with primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** (The concentration of antibody will depend on the conditions.) for 1 hr. at room temperature.
8) Wash the slide with PBS (5 min. x 3).
9) Wipe gently around the section and incubate with Histostar™ (Ms + Rb) (MBL, code no. 8460) for 30 min. at room temperature.
10) Wash the slide with PBS (5 min. x 3).
11) 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.
12) Wash the slide in water for 5 min.
13) Counterstain in hematoxylin for 1 min., wash the slide 3 times in water for 5 min. each, and then immerse the slide in PBS for 5 min.
14) Dehydrate by immersing in Ethanol 3 times for 5 min. each, followed by immersing in Xylene 3 times for 5 min. each. Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse liver ischemia model)



Immunohistochemistry in mouse liver ischemia model

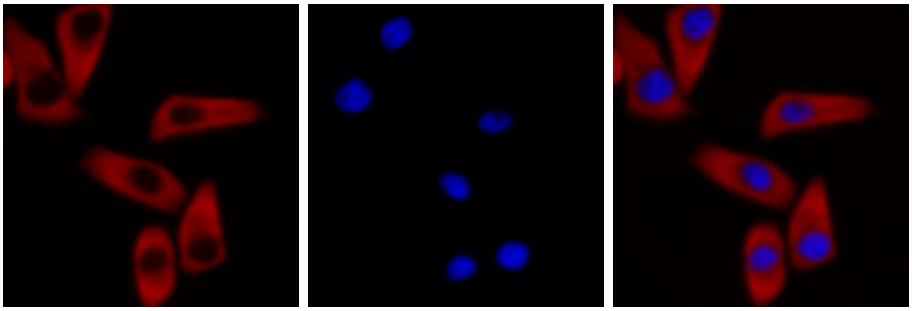
Brown: Anti-Pseudouridine mAb (MBL, code no. D347-3)
Blue: Hematoxylin

The sample was kindly provided by Dr. Takaaki Abe. (Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Biomedical Engineering)

Immunocytochemistry

1) Spread cells on a glass chamber slide, then incubate in a CO_2 incubator overnight.
2) Remove the culture supernatant by careful aspiration.
3) Wash the slide twice with PBS.
4) Fix the cells with 4% paraformaldehyde/PBS for 20 min. at room temperature (20~25°C).
5) Wash the slide 3 times with PBS.
6) Permeabilize the cells with 0.5% Triton X-100/PBS for 10 min. at room temperature.
7) Wash the slide twice with PBS.
8) Block the cells with blocking buffer (1% BSA/PBS) for 1 hr. at room temperature.
9) Tip off the blocking buffer and incubate the cells with the primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
10) Wash the slide with 0.05% Tween-20/PBS (5 min. x 3).
11) Incubate the cells with 1:1,000 Alexa Fluor® 594 Goat Anti-Mouse IgG (Thermo Fisher Scientific, code no. A-11032) diluted with blocking buffer for 1 hr. at room temperature in dark chamber.
12) Wash the slide with 0.05% Tween-20/PBS (5 min. x 3).
13) Counterstain with DAPI for 5min. and observe the slide using fluorescent microscopy.

(Positive control for Immunocytochemistry; HeLa)



Immunocytochemistry in HeLa cells
Red: Anti-Pseudouridine mAb (MBL, code no. D347-3)
Blue: DAPI