

Anti-Pseudouridine mAb

CODE No.	D347-3
CLONALITY	Monoclonal
CLONE	APU-6
ISOTYPE	Mouse IgG1 κ
QUANTITY	100 μ L, 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

<u>Immunohistochemistry</u>	1 μ g/mL (paraffin section)
<u>Immunocytochemistry</u>	1 μ g/mL
<u>RNA immunoprecipitation</u>	Not recommended

APPLICATIONS-REPORTED

<u>ELISA</u>	Reference 2) and 3)
<u>Immuno-Notern blotting</u>	Reference 1)

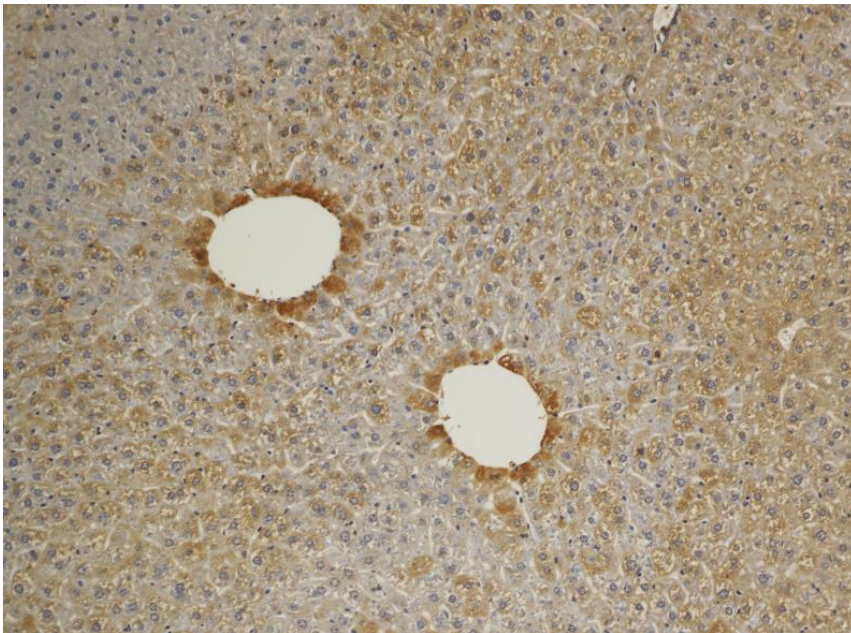
REFERENCES	1) Mishima, E., et al., <i>PLoS One</i> 10 , e0143756 (2015) [Immuno-Notern blotting]
	2) Masuda, M., et al., <i>Cancer</i> 72 , 3571-3578 (1993) [IHC-p, ELISA]
	3) Itoh, K., et al., <i>Clin. Chim. Acta</i> 181 305-315 (1989) [ELISA]
	4) Itoh, K., et al., <i>Tohoku J. Exp. Med.</i> 168 , 329-331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

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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% H₂O₂ 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

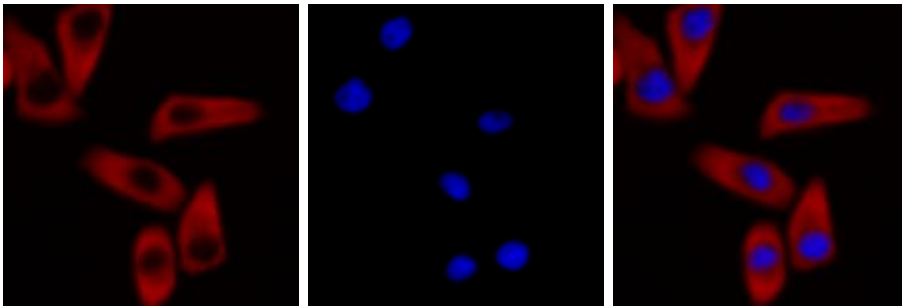
Brown: Anti-Pseudouridine mAb (MBL, code no. D347-3)
Blue: Hematoxylin

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 CO₂ 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