

# Anti-5-methylcytidine (m<sup>5</sup>C) mAb

<b>CODE No.</b>	D346-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	FMC-9
<b>ISOTYPE</b>	Mouse IgG2a $\lambda$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	KLH-conjugated 5-methylcytidine (KLH-m <sup>5</sup> C)
<b>REACTIVITY</b>	This clone reacts with 5-methylcytidine (m <sup>5</sup> C or m5Cyd), 5-methyl-2'-deoxycytidine (m5dC or m5dCyd) and 5-methylcytosine (5mC, 5-mC or m5Cyt). Please see the reference 3) for more details.
<b>FORMULATION</b>	PBS containing 50% glycerol. No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

## APPLICATIONS-CONFIRMED

<u>Immunohistochemistry</u>	10 $\mu$ g/mL (paraffin section) For nuclear staining, heat treatment or HCl treatment is required. Heat treatment: autoclave, 5 min. at 121°C in 10 mM citrate buffer (pH 6.0) HCl treatment: 30 min. at room temperature in 2 N HCl *Recommended activation; Heat treatment.
<u>Immunocytochemistry</u>	5 $\mu$ g/mL (for cytoplasmic staining) or 1 $\mu$ g/mL (for nuclear staining, HCl-treated)
<u>RNA immunoprecipitation</u>	Not recommended
<u>DNA immunoprecipitation</u>	Can be used
<u>Dot blot</u>	Can be used

<b>REFERENCES</b>	1) Seki, Y., <i>et al.</i> , <i>Dev. Biol.</i> <b>278</b> , 440-458 (2005) 2) Sakai, Y., <i>et al.</i> , <i>Cell Struct. Funct.</i> <b>26</b> , 685-691 (2001) [IHC-fr] 3) Mizugaki, M., <i>et al.</i> , <i>Biol. Pharm. Bull.</i> <b>19</b> , 1537-1540 (1996)
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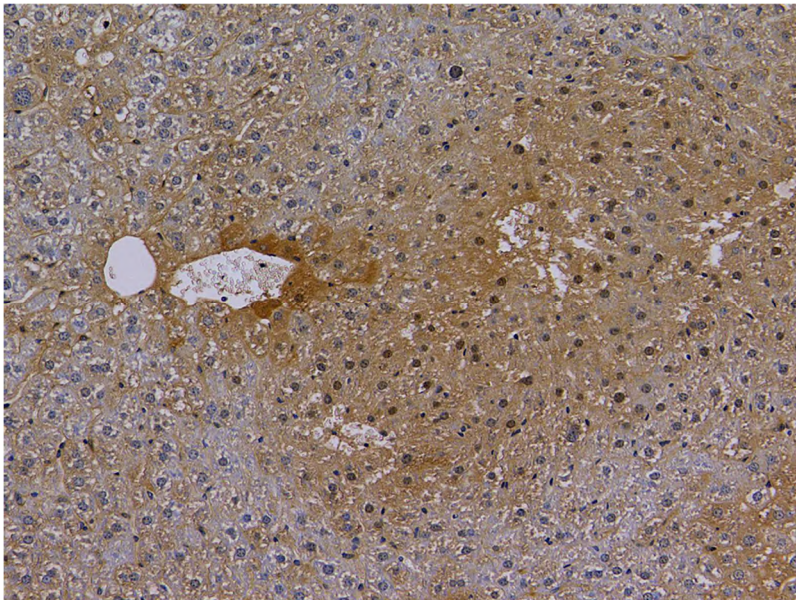
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**Immunohistochemistry for formalin fixed paraffin-embedded section**

- 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% H<sub>2</sub>O<sub>2</sub> in PBS for 10 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 (pH 7.4)] for 5 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; Ischemic model mouse liver)



***Immunohistochemistry in ischemic model mouse liver***

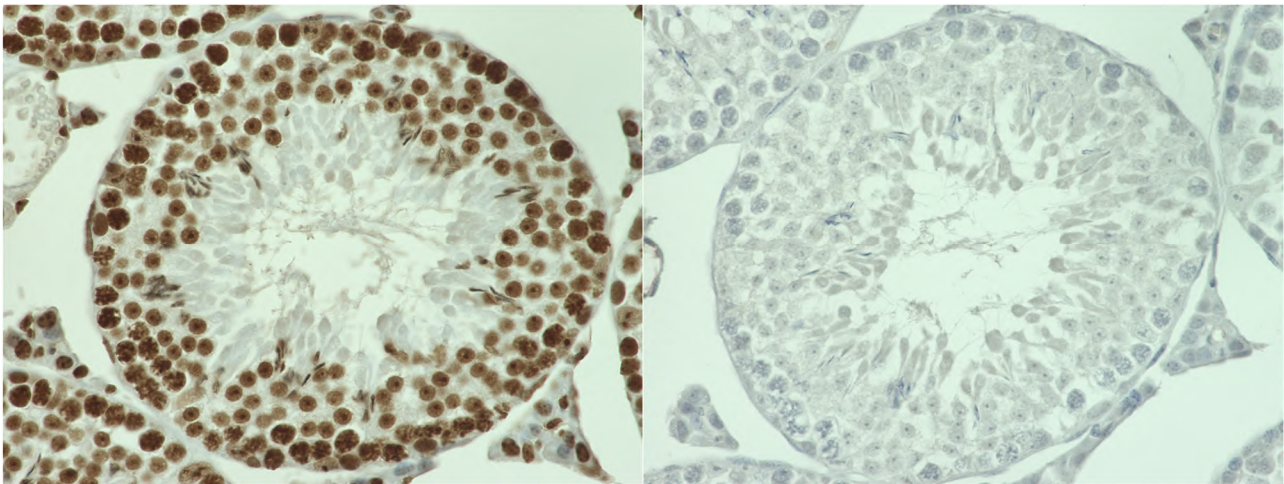
Brown: Anti-5-methylcytidine (m<sup>5</sup>C) mAb (D346-3)  
Blue: Hematoxylin

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

**Immunohistochemistry for formalin fixed paraffin-embedded section: Nuclear staining**

- 1) Deparaffinize the section with Xylene (5 min. x 3).
- 2) Wash the slides with Ethanol (5 min. x 3).
- 3) Wash the slides with PBS (5 min. x 3).
- 4) Remove the slides from PBS and heat-treat with 10 mM citrate buffer (pH 6.0) for 5 min. at 121°C using autoclave.
- 5) Let the slides cool down in citrate buffer at room temperature for 1 hr.
- 6) Remove the slides from citrate buffer and inactivate endogenous peroxidase with 3% H<sub>2</sub>O<sub>2</sub> in PBS for 10 min.
- 7) Wash the slides with PBS (5 min. x 3).
- 8) Remove the slides from PBS, wipe gently around the section and incubate 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.
- 9) 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.
- 10) Wash the slides with PBS (5 min. x 3).
- 11) Wipe gently around the section and incubate with Histostar™ (Ms + Rb) (MBL, code no. 8460) for 30 min. at room temperature.
- 12) Wash the slides with PBS (5 min. x 3).
- 13) 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.
- 14) Wash the slides in water for 5 min.
- 15) Counterstain in hematoxylin for 1 min., wash the slides 3 times in water for 5 min. each, and then immerse the slide in PBS for 5 min.
- 16) 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 testis)



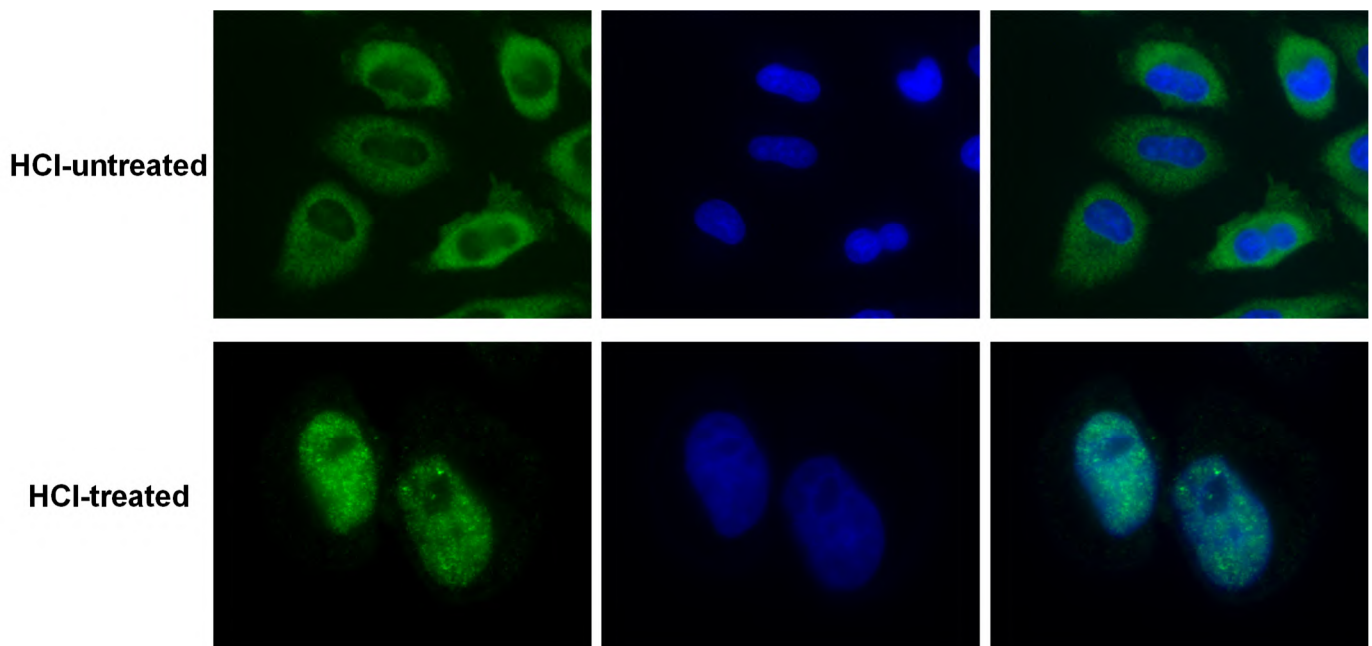
***Immunohistochemistry in mouse testis***

Left: Anti-5-methylcytidine (m<sup>5</sup>C) mAb (D346-3)  
Right: Mouse IgG2a (isotype control) (M076-3)

### **Immunocytochemistry**

- 1) Spread cells on a glass chamber slide, then incubate in a CO<sub>2</sub> incubator for one night.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Wash the slide twice with PBS.
- 4) Fix the cells with 4% paraformaldehyde (PFA)/PBS for 20 min. at room temperature (20~25°C).
- 5) Wash the slide twice with PBS.
- 6) Permeabilize the cells with 0.5% Triton X-100/PBS for 5 min. at room temperature.
- 7) Wash the slide twice with PBS. In case of cytoplasmic staining, skip to step 9).
- 8) **HCl-treatment for nuclear staining:**  
Incubate the cells with 2 N HCl for 30 min. at room temperature. Wash the slide 3 times with PBS.
- 9) Incubate the cells with blocking buffer (1% BSA/PBS) for 1 hr. at room temperature. \*10% FBS/PBS is not recommended for blocking.
- 10) 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.)
- 11) Wash the slide with 0.2% Tween-20/PBS (5 min. x 3).
- 12) Incubate the cells with 1:1,000 Alexa Fluor<sup>®</sup> 488 Goat Anti-Mouse IgG (Thermo Fisher Scientific, code no. A-11032) diluted with blocking buffer for 1 hr. at room temperature in dark chamber.
- 13) Wash the slide with 0.2% Tween-20/PBS (5 min. x 3).
- 14) Counterstain with DAPI or Hoechst33342 and observe the slide using fluorescent microscopy.

(Positive control for Immunocytochemistry; HeLa)



### ***Immunocytochemistry in HeLa cells***

Green: Anti-5-methylcytidine (m<sup>5</sup>C) mAb (D346-3)

Blue: Hoechst33342/DAPI