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Not for use in diagnostic procedures.

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Anti-5-methylcytidine (m^5C) mAb

CODE No.	D346-3
CLONALITY	Monoclonal
CLONE	FMC-9
ISOTYPE	Mouse IgG2a λ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH-conjugated 5-methylcytidine (KLH-m^5C)
REACTIVITY	This clone reacts with 5-methylcytidine (m^5C 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.
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	10 µg/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.

Immunocytochemistry	5 µg/mL (for cytoplasmic staining) or 1 µg/mL (for nuclear staining, HCl-treated)
RNA immunoprecipitation	Not recommended
DNA immunoprecipitation	Can be used
Dot blot	Can be used

REFERENCES

1) Seki, Y., *et al.*, *Dev. Biol*. **278**, 440-458 (2005)
2) Sakai, Y., *et al.*, *Cell Struct. Funct*. **26**, 685-691 (2001) [IHC-fr]
3) Mizugaki, M., *et al.*, *Biol. Pharm. Bull*. **19**, 1537-1540 (1996)

RELATED PRODUCTS

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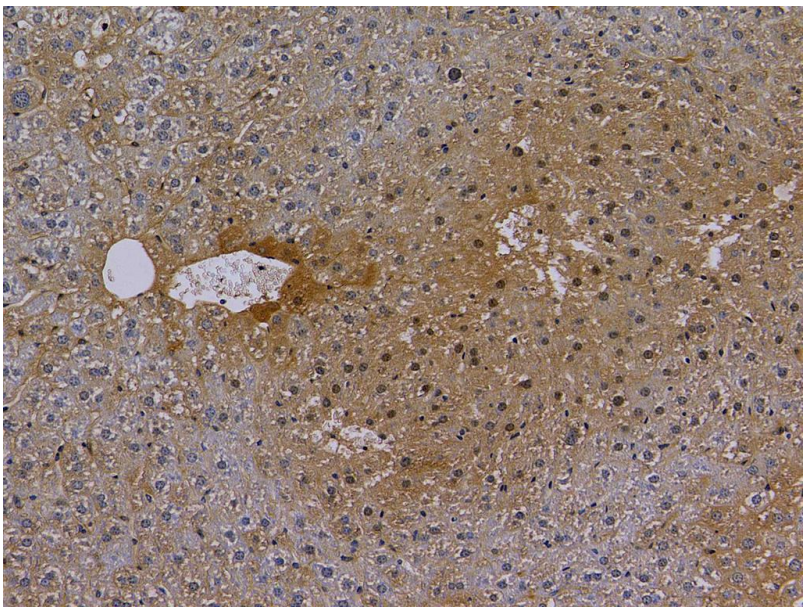
Immunohistochemistry for formalin fixed paraffin-embedded section

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_2O_2 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

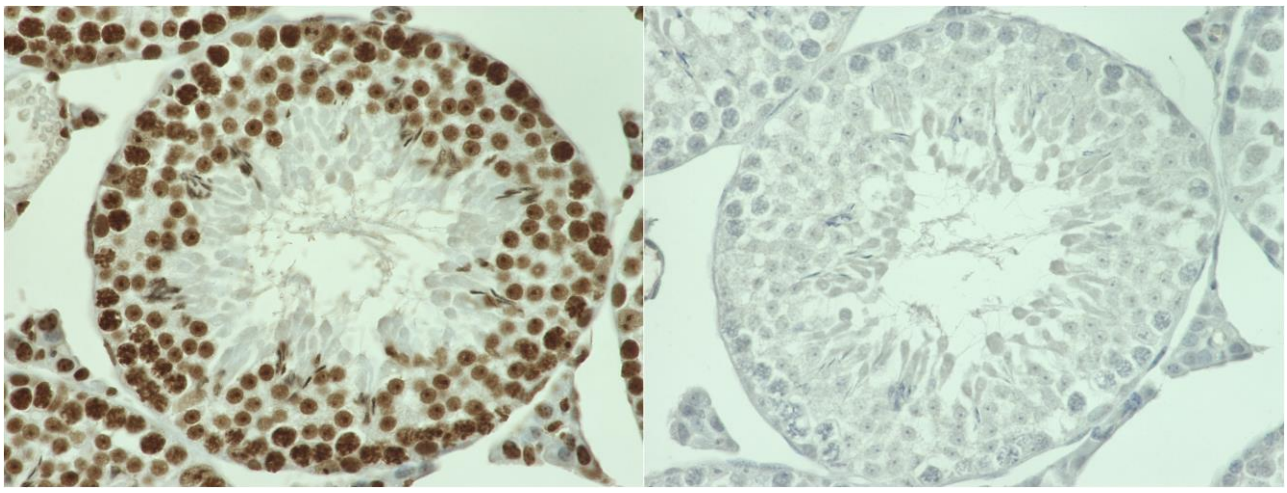
Brown: Anti-5-methylcytidine (m^5C) mAb (D346-3)
Blue: Hematoxylin

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_2O_2 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)

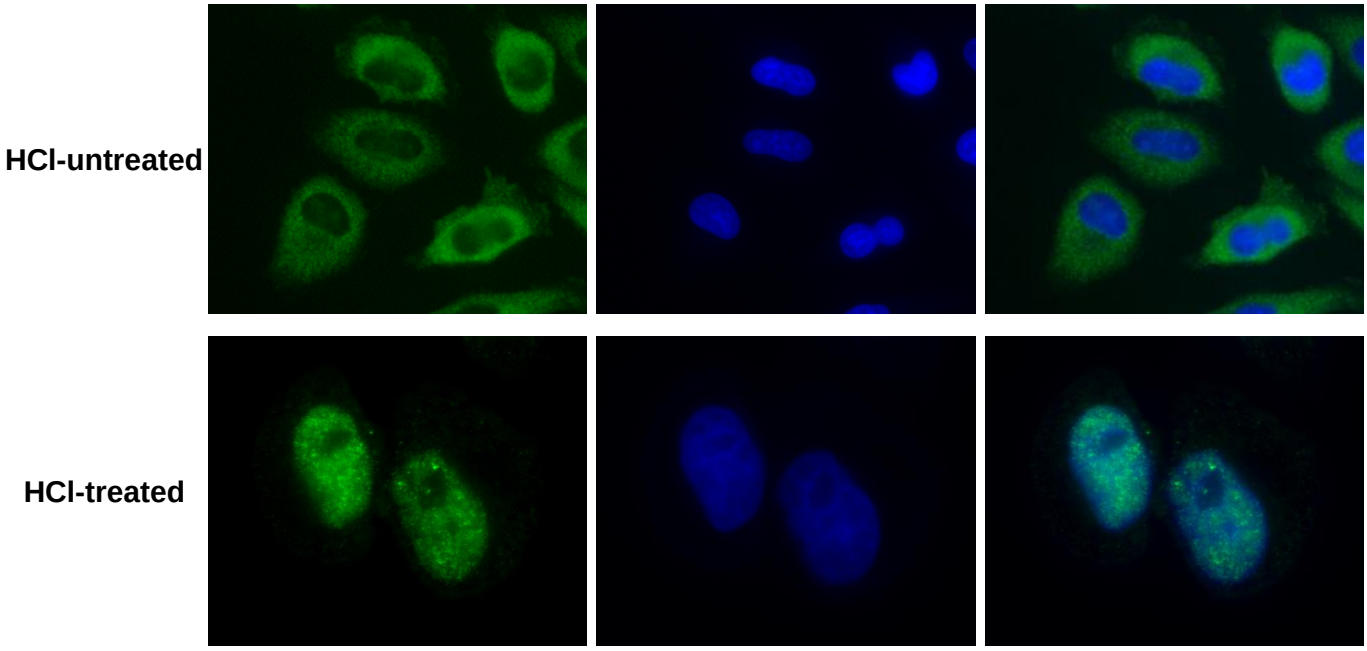


Immunohistochemistry in mouse testis
Left: Anti-5-methylcytidine (m^5C) mAb (D346-3)
Right: Mouse IgG2a (isotype control) (M076-3)

Immunocytochemistry

1) Spread cells on a glass chamber slide, then incubate in a CO_2 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® 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^5C) mAb (D346-3)
Blue: Hoechst33342/DAPI