

MONOCLONAL ANTIBODY

## Anti-Liv2 (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D118-3	Liv2	Rat IgG1	100 $\mu$ L	1 mg/mL

**BACKGROUND:** Liv2 is a fetal liver antigen that is expressed in murine embryos. Liv2 can be detected in fetal liver only during embryonic days 9.5 to 12.5, a period which corresponds to several key events in liver development. Nearly all cells in the hepatic bud were stained at E9.5, but the levels of Liv2 progressively decreased by E12.5, consistent with the numbers of hepatoblasts. Unlike other common fetal hepatic markers such as  $\alpha$ -fetoprotein or albumin, Liv2 is not a diffusible serum protein but appears to be membrane specific. Thus, Liv2 is a useful tool for identifying individual murine hepatoblasts and for studying fetal liver development.

**SOURCE:** This antibody was purified from hybridoma (clone Liv2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell PAI with WKY/Neij rat lymph nodes immunized with a E11.5 murine fetal liver lysate.

**FORMULATION:** 100  $\mu$ g IgG in 100  $\mu$ L volume of 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with Liv2 on Immunohistochemistry.

### APPLICATIONS:

Western blotting; Not tested

Immunoprecipitation; Not tested

Immunohistochemistry; 5-20  $\mu$ g/mL

Immunocytochemistry; Not tested

Flow Cytometry; 10  $\mu$ g/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

### SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissues	Not tested	Embryonic liver	Not tested
Reactivity on IHC		+	

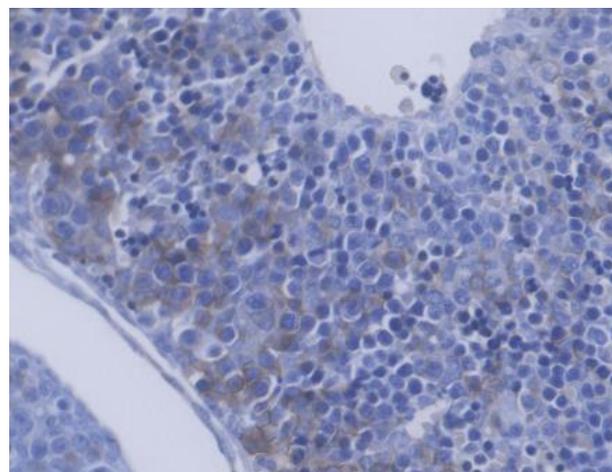
### INTENDED USE:

For research use only. Not for clinical diagnosis.

### REFERENCES:

- 1) Takashimizu, I., *et al.*, *ScientificWorldJournal* **9**, 190-9 (2009) [IHC]
- 2) Suzuki, T., *et al.*, *Mol. Cell Biol.* **26**, 6149-56 (2006) [IHC]
- 3) Nierhoff, D., *et al.*, *Hepatology* **42**, 130-139 (2005)
- 4) Watanabe, T., *et al.*, *Dev. Biol.* **250**, 332-347 (2002)

Clone Liv2 is used in these references.



**Immunohistochemical detection of Liv2 on paraffin-embedded section of mouse embryonic liver with D118-3.**

### PROTOCOLS:

#### Immunohistochemical staining for paraffin-embedded sections

- 1) Deparaffinize the sections with Xylene 3 times for 3 minutes each.
- 2) Wash the slides with Ethanol 3 times for 3 minutes each.
- 3) Wash the slides with PBS 3 times for 3 minutes each.
- 4) Remove the slides from PBS and inactivate endogenous peroxidase with 3%  $\text{H}_2\text{O}_2$  in PBS for 10 minutes.
- 5) Wash 3 times in PBS for 5 minutes each.
- 6) Remove the slides from PBS, and immerse the slides in blocking buffer (1% BSA, 20 mM HEPES, 135 mM NaCl) for 5 minutes at room temperature to block non-specific staining. Do not wash.
- 7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer as suggest in the **APPLICATIONS**.
- 8) Incubate the sections for 1 hour at room temperature.
- 9) Wash the slides 3 times in PBS for 5 minutes each.

- 10) Wipe gently around each section and cover tissues with Histostar (Rat) (MBL; code no. 8463). Incubate for 30 minutes at room temperature.
- 11) Wash the slides 3 times in PBS for 5 min. each.
- 12) Visualize by reacting for 10 minutes with Histostar DAB Substrate Solution (MBL; code no. 8469). \*DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
- 13) Wash the slides in water for 5 minutes.
- 14) Counterstain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes.
- 15) Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each. Now ready for mounting.

(Positive control for Immunohistochemistry; Mouse embryonic liver)

### **Flow cytometric analysis for cells**

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN<sub>3</sub>].
- 2) Add 200 µL of 4% paraformaldehyde (PFA) to the cell pellete after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
- 3) Wash the cells 3 times with washing buffer.
- 4) Add 200 µL of 70% ethanol to the cell pellete after tapping. Mix well, then permiabilize the cells for 30 minutes at -20°C.
- 5) Wash the cells 3 times with washing buffer.
- 6) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN<sub>3</sub> to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature (20~25°C).
- 7) Add 30 µL of the primary antibody diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
- 8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 9) Add 30 µL of 1:100 FITC conjugated anti-rat IgG (MBL: code no.IM-0827) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
- 10) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
- 11) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for flow cytometry; Mouse embryonic hepatocyte)

### **RELATED PRODUCT:**

D118-3	Anti-Liv2 (Mouse) mAb
D187-3	Anti-Dlk (Pref-1) mAb
D187-4	Anti-Dlk (Pref-1) mAb-FITC
D187-5	Anti-Dlk (Pref-1) mAb-PE
K0106-3	Anti-CD117 (c-Kit) (Human) mAb
K0105-4	Anti-CD117 (c-Kit) (Human) mAb-FITC
K0106-4	Anti-CD117 (c-Kit) (Human) mAb-FITC
566	Anti-CD117 (c-Kit) (Human) pAb
566-H	Anti-CD117 (c-Kit) (Human) pAb
D062-3	Anti-TER-119 (Mouse) mAb
D062-4	Anti-TER-119 (Mouse) mAb-FITC
D062-5	Anti-TER-119 (Mouse) mAb-PE
D225-3	Anti-LYVE-1 (Mouse) mAb
D225-5	Anti-LYVE-1 (Mouse) mAb-PE
D059-3	Anti-OSMR (Oncostatin M Receptor) (Mouse) mAb
D088-3	Anti-LECT2 (Human) mAb
D089-3	Anti-LECT2 (Human) mAb
5327	Ab-Match ASSEMBLY Human LECT2 kit
5328	Ab-Match ASSEMBLY Mouse LECT2 kit
5310	Ab-Match Universal kit