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Inspiration for Life Science

MONOCLONAL ANTIBODY

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Catalog No. RIK-MA-R58

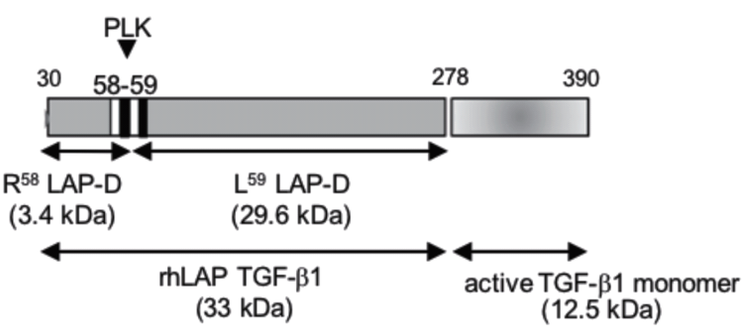
Anti TGF-β1 LAP-D (R58)

(LAP Degradates C-Terminus side cut end R58)

Background：

TGF-β is produced as a latent form in which 25 kD active TGF-β is trapped by its pro-peptide called Latency Associated Protein (LAP). Upon receiving certain stimuli, a conformational change is induced in a latent complex to release the active TGF-β from the complex. The resultant TGF-β binds to cognate signaling receptors and exerts various physiological and pathological activities. This reaction is called TGF-β activation reaction, which is known to be induced by binding of the latent complex to cell adhesion proteins such as thrombospondin and integrins, and/or by being cleaved by the action of proteases such as serine proteases, cysteine proteases, and MMPs in an organ and context-depending manner.

Kojima and his colleagues in Cellular Molecular Pathology Research Unit (currently, Center for Integrative Medical Sciences, Liver Cancer Prevention Research Unit), RIKEN, Japan identified that a serine protease, plasma kallikrein induces release and activation of TGF-β by cleaving between 58Arg-59Leu within LAP and thereby participates in the pathogenesis of the liver diseases. The anti-TGF-β1 LAP-degradates (LAP-D) antibodies are useful to investigate the molecular mechanism of TGF-β activation and its related diseases including liver fibrosis/cirrhosis and liver degeneration as tools to detect LAP-D.



Host Species:	Mouse
Form:	Liquid, PBS (pH 7.4), 0.05% NaN_3
Volume:	100 µg (1 mg/mL)
Specificity:	Recognizes C-terminus cut end of LAP degradates (LAP-D) R58 when latent TGF-β is digested with Plasma Kallikrein (PLK).
Antigen:	R58 peptide [CGQILSKLR]
Clonality:	Monoclonal (clone # 18F9-16)
Isotype:	IgG3
Applications:	Immunohistochemistry (IHC): 10 µg/mL * Optimal dilutions/concentrations should be determined by each researcher.
Purification method:	Purified from cell culture of serum-free medium by affinity column (Protein A)
Conjugation:	none
Storage condition:	Store below -20°C (below -70°C for prolonged storage) *Aliquot to avoid cycles of freeze/thaw

References:

1. LAP degradation product reflects plasma kallikrein-dependent TGF-β activation in patients with hepatic fibrosis, Hara M., Kirita A., Kondo W. et al. Springerplus. May 1; 3: 221. PMCID: PMC4033717 (2014)

* Anti TGF-β1 LAP-D (R58) was generated & licensed under RIKEN, Japan.

Example Assay Data:

1. Immunohistochemistry (IHC) Staining

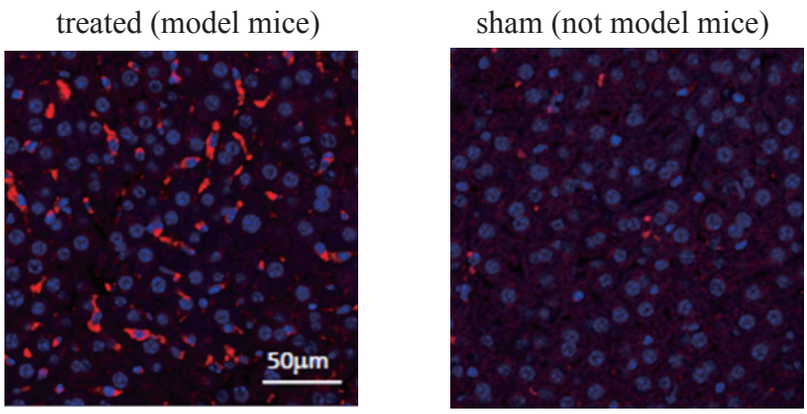


Figure 1. Immunohistochemistry (IHC) staining of liver section from a hepatitis model mice

【sample】liver, paraffin sections (4 μm) from hepatitis model mice (positive control, treated) and sham operated mice (negative control, sham)

Primary Antibody: anti TGF-β1 LAP-D (R58) antibody (10μg/mL)

1. Sectioning

Paraffin section (4 μm) / adhesive coated glass slide

2. Deparaffinizing

Xylene 5 min x 3

100% Ethanol 3 min x 2

90% Ethanol 3 min

80% Ethanol 3 min

70% Ethanol 3 min

50% Ethanol 3 min

MilliQ water

3. Staining

epitope retrieval: Target Retrieval Solution pH 9.0 [Agilent Technologies, S236784-2], microwave 98 ℃, 10 min

wash: PBST (0.1% tween 20), 5 min x 3

blocking: 10% donkey serum [ImmunoBioScience, IHR-8135] / PBST (0.1% tween 20), room temperature, 1 hr

primary antibody: anti TGF-β1 LAP-D (R58) antibody (10 μg/mL)

10% donkey serum / PBST (0.1% tween 20), 4 ℃ , overnight

wash: PBST (0.1% tween 20), 5 min x 3

secondary antibody: donkey anti-mouse IgG alexa555 [Thermo Fisher Scientific, A-31570], 1/1000, room temperature, 2 hrs

wash: PBST (0.1% tween 20), 5 min x 3

counter staining: DAPI

wash: PBST (0.1% tween 20), 5 min x 3

mounting

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