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Inspiration for Life Science

MONOCLONAL ANTIBODY

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Catalog No. RIK-MA-L59

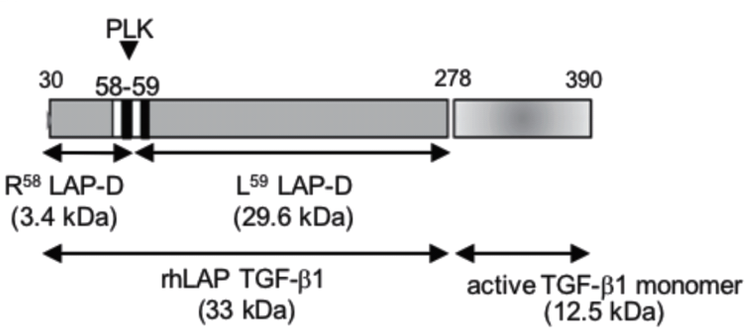
Anti TGF-β1 LAP-D (L59)

(LAP Degradates N-Terminus side cut end L59)

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, Micro-Signaling Regulation Technology 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 N-terminus cut end of LAP degradates (LAP-D) L59 when latent TGF-β is digested with Plasma Kallikrein (PLK).
Antigen:	L59 peptide [LASPPSQGEVPGGC]
Clonality:	Monoclonal (clone # 6D6)
Isotype:	IgG1
Applications:	Western Blot: 2-10 µg/mL ELISA (Enzyme-Linked ImmunoSorbent Assay): 20 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 G)
Conjugation:	none
Storage condition:	Store below -20°C (below -70°C for prolonged storage) *Aliquot to avoid cycles of freeze/thaw

* Anti TGF-β1 LAP-D (L59) was generated & licensed under RIKEN, Japan.

References:

1. Hara M., Kirita A., Kondo W. et al. (2014) LAP degradation product reflects plasma kallikrein-dependent TGF-β activation in patients with hepatic fibrosis, *Springerplus*. **3**: 221. PMID: 24877031

2. Hara M., Inoue I., Yamazaki Y. et al. (2015) L^{59} TGF-β LAP degradation products serve as a promising blood biomarker for liver fibrogenesis in mice, *Fibrogenesis Tissue Repair*. **8**: 17. PMID: 26379781

Example Assay Data:

1. Western Blotting

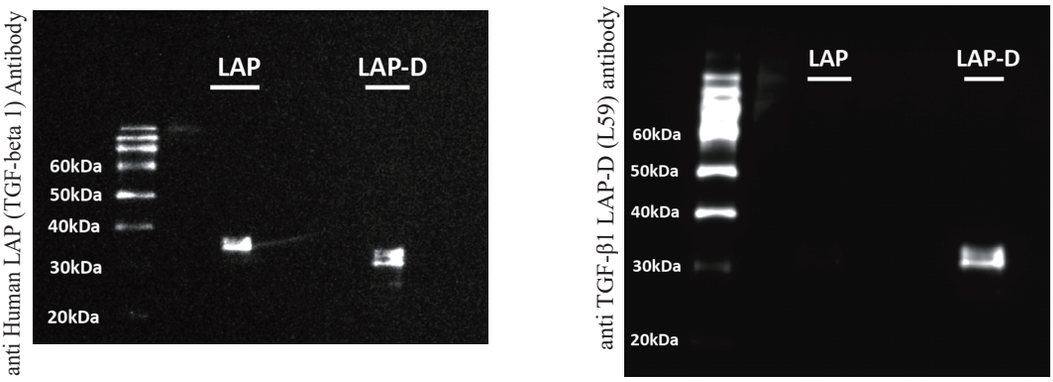


Figure 1. Western Blotting result with anti TGF-β1 LAP-D (L59) antibody

【sample】 uncut human recombinant LAP (R&D Systems, 246-LP-025) (LAP), PLK digested human recombinant LAP (LAP degradates, LAP-D) [10 ng/lane]
Antibody:
(left figure) anti Human LAP (TGF-beta 1) Antibody (R&D Systems, AF-246-NA, 0.1 µg/mL), Peroxidase AffiniPure F(ab')$_2$ Fragment Rabbit Anti-Goat IgG (H+L)（Jackson ImmunoResearch Laboratories, 305-036-045, 0.08 µg/mL)
(right figure) anti TGF-β1 LAP-D (L59) antibody (10 µg/mL), Peroxidase AffiniPure F(ab')$_2$ Fragment Goat Anti-Mouse IgG (H+L)（Jackson ImmunoResearch Laboratories, 115-036-062, 0.08 µg/mL)
Detection: Pierce® ECL Plus Western Blotting Substrate (ThermoFischer Scientific, NCI32132JP)
* anti TGF-β1 LAP-D (L59) antibody specificity has been confirmed by detection of LAP-D [31 kDa]

2. ELISA (Enzyme-Linked ImmunoSorbent Assay)

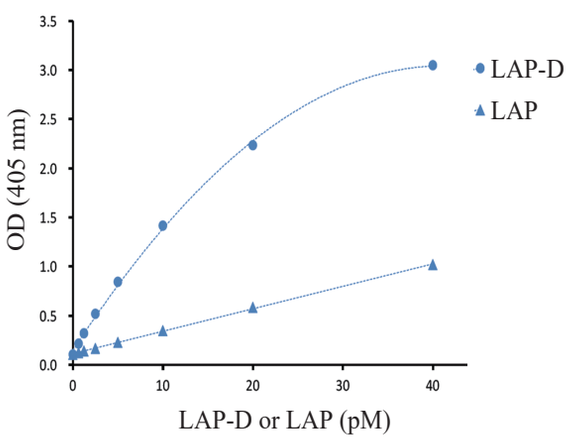


Figure 2. Sandwitch ELISA with anti TGF-β1 LAP-D (L59) antibody and anti Human LAP TGF-beta 1 Biotinylated antibody

【sample】 PLK digested human recombinant LAP (L59 LAP degradates, LAP-D), uncut human recombinant LAP (LAP)
Coating Antibody: anti TGF-β1 LAP-D (L59) antibody [20 µg/mL]
Detection Antibody: anti Human LAP TGF-beta 1 Biotinylated Antibody（R&D Systems, BAM2462, 0.5 µg/mL)
Detection: Streptavidin-AP (Jackson ImmunoResearch Laboratories, 016-050-084, 0.05 µg/mL)
* This ELISA system detected uncut human recombinant LAP, which showed c.a. 1 OD at 40 pM, while LAP-D showed c.a. 3 OD at 40 pM, giving siginificant differences. This result proves the specificity of anti TGF-β1 LAP-D (L59) antibody.

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