

# SMP30 (Senescence Marker Protein 30) Regucalcin Gluconolactonase (GNL) WesternBlot - ImmunoStain Kit

Catalog Number: ROIKO1-EX

Kit component

Antibody: Rabbit anti SMP30 • GNL antibody 0.1 mL

10 mM Tris (pH 7.4), 0.14 M NaCl

This kit does not contain NaN<sub>3</sub>

Specimen: SMP30 • GNL Knockout Mouse Liver 2 slides

Wild type Mouse Liver 2 slides

Tissue extract : SMP30 • GNL Knockout Mouse Liver 30 μL

(Protein concentration 0.4 mg/mL) Wild type Mouse Liver  $30 \mu L$  (Protein concentration 0.4 mg/mL)

Storage and Stability: -20 °C, 2 years

### [Antibody]

SMP30 (Senescence Marker Protein 30) IgG

Gluconolactonase (GNL) IgG

Regucalcin IgG

★ SMP30, Gluconolactonase and Regucalcin are all identical protein.

Rabbit Polyclonal Antibody (Purified IgG Fraction)

Volume: 0.1 mL

Antigen: Rat SMP30 purified from rat liver, Molecular weight 34 kDa

Host : Rabbit

Supplied As: IgG fraction purified from rabbit serum.

Prepared in 10 mM Tris (pH 7.4), 0.14 M NaCl.

Storage and Stability: -20 °C, 2 years

Tested applications : • Immunofluorescence (1:100-1:500 dilution)

• immunohistochemistry (1:100-1:500 dilution)

Western Blot (1:1,000-1:3,000 dilution)

Cross Reactivity: Cross reacts with Human, Mouse and Rat SMP30.

Not yet tested in other species.



# [Sample Preparation for Western blot]

SMP30 • GNL Knockout Mouse Liver 30  $\mu$ L (Protein concentration 0.4 mg/mL) Wild type Mouse Liver 30  $\mu$ L (Protein concentration 0.4 mg/mL)

- 1. Add 30 µl SDS-PAGE Lysis Buffer.
- 2. Boil at 95°C for 5 minutes and cool on ice.
- 3. Centrifuge at 10,000 rpm for 5 minutes and transfer the supernatant to a fresh tube.
- 4. Load the sample 10 μL (Protein 2 μg) per lane.

### 2X SDS-PAGE Lysis Buffer

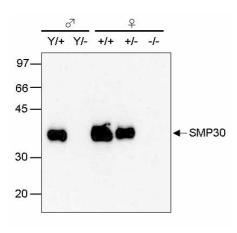
125 mM Tris-HCl, pH 6.8

**4% SDS** 

10% 2-mercaptoethanol

20% glycerol

0.01% bromophenol blue (BPB)



## [Western Blot Analysis]

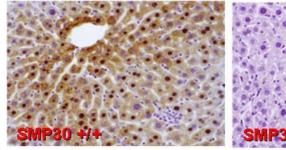
Each lanes: Mouse Liver Extract

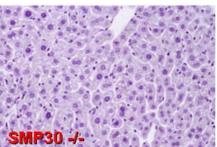
• Wild type Mouse : SMP30Y/+ and SMP30+/+

• SMP30 Knockout Mouse : SMP30Y/- and SMP30-/-

• Heterozygous Mouse : SMP30+/-

SMP30 • GNL antibody at 1:1,000 dilution used.

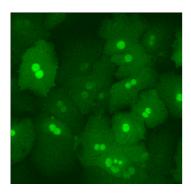




### [Immunohistochemical staining]

Mouse liver stained with SMP30 • GNL antibody at 1:300 dilution and developed by 3,3'-diaminobenzidine. Nucleus and cytoplasm of wild type (SMP30+/+) mice stained, but not stained in liver from SMP30 knockout (SMP30-/-) mice.





# [Immunofluorescence staining]

Primary cultured mouse hepatocytes stained with SMP30 • GNL antibody at 1:200 dilution. Nucleus and cytoplasm stained in green.

#### References:

- 1. Ishigami, A. et al., Senescence marker protein-30 knockout mouse liver are highly susceptible to TNF-alpha- and Fas-mediated apoptosis. *Am. J. Pathol.* 161 1273-1281 (2002)
- 2. Ishigami, A. et al., Nuclear localization of senescence marker protein-30 (SMP30) in cultured mouse hepatocytes and its homology to RNA polymerase. *Biosci. Biotechnol. Biochem.* 67 158-160 (2003)
- Kondo, Y. et al., Senescence Marker Protein 30 Functions as Gluconolactonase in L-Ascorbic Acid Biosynthesis and Its Knockout Mice Are Prone to Scurvy. *Proc. Nat. Acad. Sci. USA* <u>103</u> 5723-5728 (2006)
- 4. Sato, T. et al., Senescence Marker Protein-30 Protects Mice Lungs from Oxidative Stress, Aging and Smoking. *Am. J. Respir. Crit. Care Med.* <u>174</u> 530-537 (2006)

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TOYO EKIMAE BLDG, 2-20,TOYO 2CHOME,KOTO-KU,TOKYO 135-0016,JAPAN TEL: +81-3-5632-9617 FAX: +81-3-5632-9618 e-mail: export@cosmobio.co.jp www.cosmobio.com