Human Angiopoietin-Like 3 (ANGPTL3) Rabbit Polyclonal Antibody

Cat# CY-P1019

50 µg (1 mg/ml x 50 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB	H	60 kDa	Rabbit IgG

Background

The ANGPTL3 (Angiopoietin Like 3) gene was identified by searching an EST database for signal sequences and amphipathic helices of angiopoietins (1). The deduced 460-amino acid ANGPTL3 protein has the characteristic structure of angiopoietins: a signal peptide, an extended helical domain predicted to form dimeric or trimeric coiled-coils, a short linker peptide, and a globular fibrinogen homology domain. ANGPTL3 does not contain the characteristic calcium-binding motif found in other angiopoietins. Camenisch et al. (2002) determined that ANGPTL3 binds to human vascular endothelial cells; however, it does not bind to the Tie2 receptor, which is utilized by other members of the angiopoietin family to regulate blood vessel formation (2). Binding induced integrin alpha-5/beta-3-dependent haptotactic endothelial cell adhesion and migration, and stimulated signal transduction pathways characteristic for integrin activation. ANGPTL3 also induced angiogenesis in the rat corneal assay.

ANGPTL3 gene was also identified as a responsible gene for inherited hypolipidemia in KK/San mice (3). In vitro analysis of recombinant protein revealed that Angptl3 directly inhibits lipoprotein lipase (LPL) activity. From these data, Shimizugawa et al. (2002) concluded that Angptl3 regulates VLDL triglyceride levels through the inhibition of LPL activity (4).

Specificity/Sensitivity: Human Angiopoietin-like 3 (ANGPTL3) Antibody detects endogenous levels of human ANGPTL3 protein. The antibody does not cross-react with other angiopoetin-Like proteins and angiopoietin proteins.

Source/Purification: Polyclonal antibody is produced by immunizing rabbit with a synthetic human ANGPTL3 peptide derived from the coiled-coil domain 2 sequence of human ANGPTL3. IgG is purified by peptide-affinity chromatography.

Recommended Antibody Dilutions: Western blotting: 1-2 µg/mL

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western **IP:**Immunoprecipitation **IHC:**Immunohistochemistry **IC:**Immunocytochemistry **F:**Flow cytometry **E:**ELISA **FP:**Fluorescence Polarization assay
Species Cross-Reactivity Key: H:human **M:**mouse **R:**rat **Hm:**hamster **Mk:**monkey **Mi:**mink **C:**chicken **X:***Xenopus* **Z:**zebra fish **All:**all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Background References:

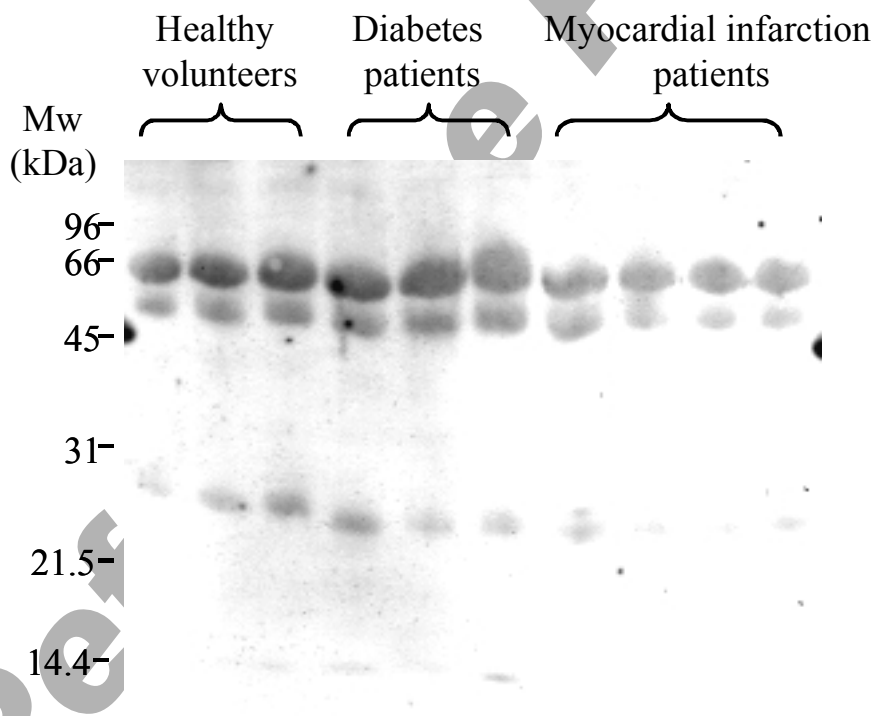
1. Conklin, D.; Gilbertson, D.; Taft, D. W.; Maurer, M. F.; Whitmore, T. E.; Smith, D. L.; Walker, K. M.; Chen, L. H.; Wattler, S.; Nehls, M.; Lewis, K. B.: Identification of a mammalian angiopoietin-related protein expressed specifically in liver. Genomics 62: 477-482, 1999.

2. Camenisch, G; Pisabarro, M. T.; Sherman, D.; Kowalski, J.; Nagel, M.; Hass, P; Xie, M.-H.; Gurney, A.; Bodary, S.; Liang, X. H.; Clark, K.; Beresini, M; Ferrara, N.; Gerber, H.-P. : ANGPTL3 stimulates endothelial cell adhesion and migration via integrin alpha-v-beta-3 and induces blood vessel formation in vivo. J. Biol. Chem. 277: 17281-17290, 2002.

3. Koishi, R.; Ando, Y.; Ono, M.; Shimamura, M.; Yasumo, H.; Fujiwara, T.; Horikoshi, H.; Furukawa, H. : Angptl3 regulates lipid metabolism in mice. Nature Genet. 30: 151-157, 2002.

4. Shimizugawa, T.; Ono, M; Shimamura, M; Yoshida, K.; Ando, Y.; Koishi, R.; Ueda, K.; Inaba, T; Minekura, H; Kohama, T.; Furukawa, H. : ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. J. Biol. Chem. 277: 33742-33748, 2002.

Fig.1 Western blot analysis of human ANGPTL3 in human sera



Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent; for 20 mL, add 2 mL 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten second exposure should indicate the proper exposure time.

Immunoprecipitation Followed by Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Cell Lysis Buffer (1X): 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Glycerolphosphate, 1 mM Na_3VO_4, 1 µg/ml Leupeptin
Note: We recommend adding 1 mM PMSF before use.
Protein A Agarose Beads: Add 5 mL of 1X PBS to 1.5 g of Protein A Agarose Beads. Shake 2 hours at 4°C; spin down. Wash the pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)
3X SDS Sample Buffer: 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30%, glycerol, 150 mM DTT, 0.03% w/v bromophenol blue.
Transfer Buffer:25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk. For 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): For 1 liter of 10X TBS, use 24.2 g Tris base and 80 g NaCl. Adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.05% Tween-20 with 5% nonfat dry milk. For 20 mL, add 2 ml 10X TBS to 18 mL water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 10 µL Tween-20 (100%).
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 mL 1X ice-cold Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm^2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate 4 times for 5 seconds each on ice.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at –80°C.

Immunoprecipitation

1. Take 200 µL cell lysate and add primary antibody (2-4 µg; incubate with gentle rocking for 2 hrs or overnight at 4°C.
2. Add protein A agarose beads (20 µL of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet 3 times with 500 µL of 1X Cell Lysis Buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 µL 3X SDS Sample Buffer. Votex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes.
6. Load the sample (15–30 µL) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

Related Products

*** Human Adiponectin Rabbit Polyclonal antibody: Cat# CY-P1017**
*** Mouse Adiponectin Rabbit Polyclonal antibody: Cat# CY-P1018**
*** Human ANGPTL4 Rabbit Polyclonal antibody: Cat# CY-P1021**
*** Mouse ANGPTL4 Rabbit Polyclonal antibody: Cat# CY-P1022**

PRODUCED BY

CycLex Co., Ltd.
1063-103 Terasawaoka
Ina, Nagano 396-0002
Japan
Fax: +81-265-76-7618
e-mail: info@cyclex.co.jp
URL: http://www.cyclex.co.jp