

# Mouse Angiopoietin-Like 4 (ANGPTL4) Rabbit Polyclonal Antibody

Cat# CY-P1022

50 µg (1 mg/ml x 50 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB	M	72 kDa 32 kDa	Rabbit IgG

## Background

The angiopoietin-like 4 (ANGPTL4, FIAF, PGAR, HFARP) was previously identified as a novel adipocytokine that was up-regulated by fasting, by peroxisome proliferators-activated receptor agonists, and by hypoxia (1-3).

The 410-amino acid, 60-kD human ANGPTL4 protein, which shares 75% amino acid identity with the human protein, is a member of the angiopoietin family of secreted proteins and bears highest similarity to angiopoietin-2 (ANGPT2). Like other members of this family, ANGPTL4 contains a predicted coiled-coil quaternary structure, and the authors hypothesized that ANGPTL4 may form multimeric or other higher-order structures. ANGPTL4 has a secretory signal peptide, 3 potential N-glycosylation sites, and 4 cysteines that may be available for intramolecular disulfide bonding. ANGPTL4 protein can be detected in various tissues and in blood plasma, suggesting that ANGPTL4 has an endocrine function. Its plasma abundance is increased by fasting and decreased by chronic high fat feeding. The data suggest that FIAF represents a novel endocrine signal involved in the regulation of metabolism, especially under fasting conditions (4).

**Specificity/Sensitivity:** Mouse Angiopoietin-like 4 (ANGPTL4) Antibody detects endogenous levels of mouse ANGPTL4 protein. The antibody does not cross-react with other angiopoietin-Like protein and angiopoietin proteins.

**Source/Purification:** Polyclonal antibody is produced by immunizing rabbit with a synthetic mouse ANGPTL4 peptide derived from the amino-terminal sequence of mouse ANGPTL4. 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.

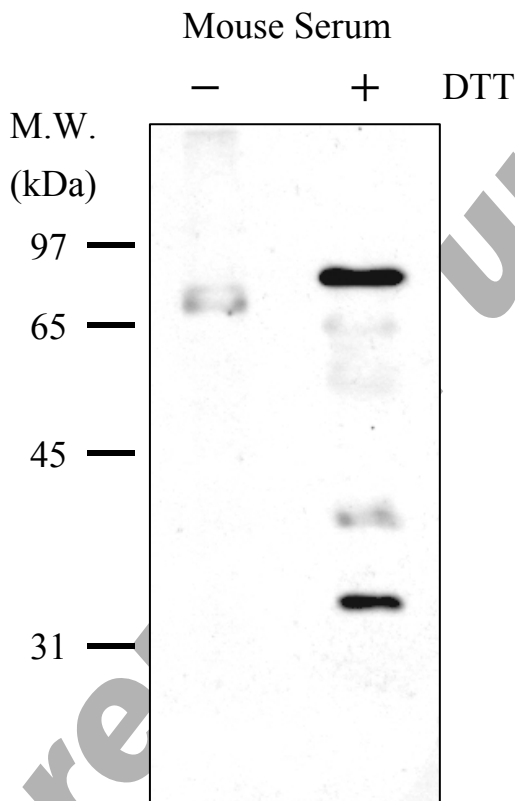
**Applications Key:** WB:Western IP:Immunoprecipitation IHC:Immunohistochemistry IC:Immunocytochemistry F:Flow cytometry E:ELISA FP:Fluorescence Polarization assay

**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.

**General References:**

1. J Biol Chem. 275(37):28488-93, 2000
2. Mol Cell Biol. 20(14):5343-9, 2000
3. J Mol Cell Cardiol. 34(7):765-74, 2002
4. J Biol Chem. 279(33):34411-20, 2004

**Fig. 1. Western blot analysis of mouse ANGPTL4**



## 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 cm<sup>2</sup> 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<sup>2</sup>) 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.

**Related Products**

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

**PRODUCED BY**

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

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