ab285296 – Mouse/Rat ANGPTL3 Serum ELISA Kit

For the quantitative measurement of Mouse/Rat ANGPTL3 in serum and plasma.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285296

Storage and Stability

Reagents must be stored at 4°C when not in use. Bring reagents to room temperature before use. Do not expose reagents to temperatures greater than 25°C.

Materials Supplied

Item	Quantity	Storage Condition
ELISA Plate	6 x 16 strips	+4°C
Wash Buffer (10X)	2 x 30 mL	+4°C
10X ELISA Buffer	2 x 30 mL	+4°C
Detection antibody	30 µL	+4°C
HRP-Streptavidin Conjugate	1 vial	+4°C
Mouse ANGPTL3 Standard (lyophilized)	1 vial	+4°C
TMB Substrate Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
Plate Sealers	2	+4°C
Gel Minibags	2	+4°C

Reagent Preparation

Prepare just the appropriate amounts for the assay.

Detection Antibody: Dilute 1:500 in ELISA Buffer 1x.

Δ Note: *The diluted Detection Antibody is not stable and cannot be stored.*

HRP-Streptavidin Conjugate: Reconstitute with 200 µl of ELISA Buffer 1X. Prepare aliquots and store them at -20°C. Avoid freeze/thaw cycles. Dilute the reconstituted HRP-Streptavidin Conjugate to the working concentration by adding 50 µl in 10 mL of ELISA Buffer 1X (1:200).

Δ Note: *The diluted HRP-Streptavidin Conjugate is not stable and cannot be stored.*

Wash Buffer: Dilute 10X Wash Buffer 1:9 with dH_2O to obtain 1X Wash Buffer.

ELISA Buffer: Dilute 10X ELISA Buffer 1:9 with dH_2O to obtain 1X ELISA Buffer.

Standard Preparation

- Reconstitute mouse ANGPTL3 Standard with 1 mL of dH_2O to produce a stock solution (2 ng/ml). Mix the Stock solution to ensure complete reconstitution.
- Allow to sit for a minimum of 15 minutes. The reconstituted standard should be aliquoted and stored at -20°C.
- Prepare Standard Curve using 2-fold serial dilutions with 1X ELISA Buffer
- Suggested standard points are: 1, 0.5, 0.25, 0.125, 0.063, 0.031, 0.016 ng/mL

Sample Preparation

Samples to be assayed immediately, otherwise samples must be aliquoted and stored at -20°C. Avoid multiple freeze-thaw cycles.

Serum: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1000 x g.

Plasma: Collect using heparin, EDTA or citrate as an anticoagulant. Centrifugation for 15 minutes at 1000 x g within 30 minutes of collection.

Δ Note: *Serum, Plasma, Urine or Cell Culture Supernatant must be diluted in Diluent 1X. Samples containing visible precipitates must be clarified before use. As a starting point, 1/4000 dilution of mouse samples and 1/400 dilution of rat samples are recommended.*

Δ Note: *If sample values fall outside of the detection range, a lower/higher dilution may be required.*

Assay Protocol

Δ Note: *It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.*

1. Determine the number of 16-well strips needed for assay and insert them into the frame for current use. The extra strips should be resealed in the foil pouch and can be stored at 4°C for up to 1 month.
2. Add 100 µL of the Standards, and Samples into the appropriate wells in duplicate.
3. Cover the plate with plate sealer and incubate for 1 hr at 37°C.
4. Aspirate and wash 3 times with 300 µL of 1X Wash Buffer. Completely remove the liquid after the last wash.
5. Add 100 µL detection antibody into each well.
6. Cover plate with plate sealer and incubate for 1 hr at 37°C.
7. Aspirate and wash 3 times with 300 µL of 1X Wash Buffer. Completely remove the liquid after the last wash.
8. Add 100 µL of diluted HRP Labeled Streptavidin into each well.
9. Cover plate with plate sealer and incubate for 1 hr at 37°C.
10. Remove plate from 37°C, aspirate and wash 5 times with 300 µL of 1X Wash Buffer.
11. After last wash, tap inverted plate on a stack of paper towels. Completely remove the liquid after the last wash.
12. Add 100 µl of TMB Substrate Solution to each well.
13. Allow the colour to develop at room temperature in the dark for 15 minutes.
14. Stop the reaction by adding 100 µL of Stop Solution to each well.

Δ Note: *Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.*

Caution: Stop Solution is a Corrosive Solution

15. Measure the OD at 450 nm in an ELISA plate reader within 30 minutes.

Calculation

1. Average the duplicate readings for each standard, controls and sample and subtract the average blank value (obtained with the 0 ng/ml point).

2. Generate a Standard Curve by plotting the average absorbance on the horizontal (X) axis vs. the corresponding concentration (µg /ml) on the vertical (Y) axis.
3. Calculate the Test Sample ANGPTL3 concentrations by interpolation of the Standard Curve regression curve as shown below in the form of a quadratic equation.
4. If the Test Samples were diluted, multiply the interpolated values by the dilution factor to calculate the corrected mouse or rat ANGPTL3 concentrations.

Performance Characteristics

Intra-assay precision: Six samples of known concentrations of mouse and rat ANGPTL3 were assayed in replicates 10 times to test precision within an assay.

Samples	Mean	SD	CV (%)	n
1	512.29	19.07	3.72	10
2	481.98	17.94	3.72	10
3	509.66	25.32	4.97	10
4	391.49	23.03	5.88	10
5	502.42	21.19	4.22	10
6	818.34	37.33	4.56	10

Inter-assay precision: Five samples of known concentrations of mouse and rat ANGPTL3 were assayed in 5 separate assays to test precision between assays.

Samples	Mean	SD	CV (%)	n
1	555.19	32.61	5.87	5
2	439.08	27.65	6.3	5
3	487.63	38.82	7.96	5
4	63.78	4.32	6.78	5
5	778.45	39.49	5.07	5

Recovery: When samples (serum) are spiked with known concentrations of mouse and rat ANGPTL3, the recovery averages 100% (range from 90% to 110%).

Samples	Average Recovery (%)	Range (%)
1	93.85	90-100
2	98.63	95-105
3	109.56	105-110

Expected values: ANGPTL3 levels range in mouse samples from 50 to > 1,000 ng/mL. ANGPTL3 levels range in rat samples from 10 to > 150 ng/mL.

Technical Hints and Limitations:

- It is recommended that all standards, controls and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100 µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 8-well strips, DO NOT let the strips DRY at any time during the assay.
- Keep TMB Substrate Solution protected from light.
- The Stop Solution consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)