

MOUSE/RAT AUTOTAXIN/ENPP2 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
MOUSE OR RAT AUTOTAXIN/ENPP2
CONCENTRATIONS IN SERUM



ALWAYS REFER TO LOT SPECIFIC
PROTOCOL PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN
DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	MOUSE/RAT AUTOTAXIN/ENPP2 ELISA
Catalog No.	SK00526-01
Formulation	96 T
Lot No.	20114628
Standard range	0.125 - 8 ng/mL
Sensitivity	30 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum
Specificity	Mouse or Rat Autotaxin
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 4 months. More information see page 2
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCIERA BIOSCIENCE, INC.
 2348 Walsh Ave., Suite C
 Santa Clara, CA 95051
 USA
 Tel: (408) 982 0300
 Fax: (408) 982 0301
 Email: Sales@AvisceraBioscience.com
 Website: www.AvisceraBioscience.com

DESCRIPTION

This Mouse/Rat Autotaxin/ENPP2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural mouse or rat autotaxin from serum in a sandwich ELISA format.

This immunoassay contains recombinant Autotaxin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural mouse or rat Autotaxin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for Autotaxin. The capture antibody can bind to the Autotaxin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against Autotaxin is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of Autotaxin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_Each laboratory must determine the optimal dilution factors for the samples being assayed.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Autotaxin Microplate – 96 well microplate coated with an antibody specific for mouse Autotaxin.	526-01-01	1 plate
Autotaxin Standard – 32 ng/vial of lyophilized recombinant Autotaxin.	526-01-02	1 vial
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of lyophilized biotinylated antibody against Autotaxin.	526-01-03	1 vial
Positive Control – one vial of lyophilized recombinant Autotaxin.	526-01-04	1 vial
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	SAHRP	1 vial
Dilution Buffer – 40 mL of buffered solution with preservative.	DB01	1 bottle
Antibody Diluent Solution – 12 mL of buffered solution with preservative.	DB32	1 bottle
HRP Diluent Solution – 12 mL of buffered solution with preservative.	DB08C	1 bottle
Wash Buffer – 25 mL of 20-fold concentrated buffered surfactant with preservative.	WB01	1 bottle
TMB Substrate Solution – 11 mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution – 11 mL of 0.25M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece
Plastic Pouch	P01	1 piece

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 4 months. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent solution should be stored at -20° C. Streptavidin-

HRP Conjugate and TMB Substrate Solution should be stored only at 2 - 8° C.
Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Mouse EDTA Plasma or Rat EDTA plasma samples CAN NOT BE Detected by this ELISA Kit.

SAMPLE PREPARATION

Mouse or rat Serum samples may need 2~ 4 Fold dilutions. If the sample concentration assayed exceeds that of the highest standard, a 2- or 4-fold dilution is suggested. A suggested 2-fold dilution is 125 µL sample + 125 µL Dilution Buffer. A suggested 4-fold dilution is 60 µL sample + 180 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

REAGENT PREPARATION

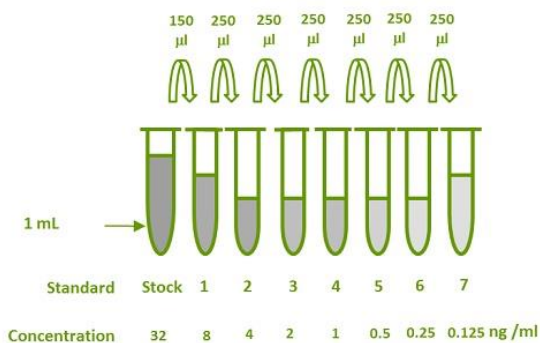
Bring all reagents to room temperature before use.

Wash Buffer – If crystals have formed in the concentrate, warm bottle in a water bath until the

crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate 20X into 475 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

Autotaxin Standard – Reconstitute the Autotaxin standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 32 ng/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1.0 mL	32 ng/mL
# 1	150µL of stock	450µL	8 ng/mL
# 2	250µL of 1	250µL	4 ng/mL
# 3	250µL of 2	250µL	2 ng/mL
# 4	250µL of 3	250µL	1 ng/mL
# 5	250µL of 4	250µL	0.5 ng/mL
# 6	250µL of 5	250µL	0.25 ng/mL
# 7	250µL of 6	250µL	0.125 ng/mL



Positive Control - Reconstitute the Positive Control with 1.0 mL Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Antibody Diluent Solution (DB32) to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Freshly Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate – Freshly Pipette 11.88 mL of **HRP Diluent Solution (DB08C)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Protect from light. DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Add 100 µL per well of **Dilution Buffer** to Blank wells.
3. Add 100 µL per well of **Standard Dilutions** (in reverse order of serial dilution from #7 - S), **samples**, or **positive control**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
5. Add 100 µL per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration and wash as in step 4.
7. Add 100 µL per well of **Streptavidin-HRP Conjugate working solution**. Cover with plate sealer and incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration and wash as in step 4.
9. Add 100 µL per well of **Substrate Solution**. Incubate for 9-13 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read plate using a microplate reader set to 450 nm within 3 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of

generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

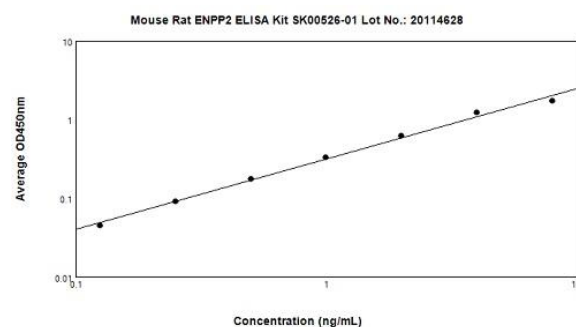
This assay recognizes both natural and recombinant mouse Autotaxin. The data indicates that rat serum samples can bind to the antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. This means rat serum samples cross-react with mouse Autotaxin ELISA kit. There is no cross-reaction with mouse Vaspin.

TYPICAL DATA

This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

AUTOTAXIN (NG/ML)	CORRECTED (450NM)
Blank	0 (0.119)
0.125	0.045
0.25	0.090
0.5	0.175
1	0.329
2	0.613
4	1.219
8	1.701
32 (optional)	2.595

- Lot: 20114628
- Positive control: 1 - 4 ng/mL
- Standard Curve fit by log-log



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL per well of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 90 minutes on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Streptavidin-HRP Conjugate working solution. Cover with plate sealer and incubate 45 minutes on microplate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate 10-11 min on microplate shaker at RT. Protect from light.
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 3 minutes.

Our research data indicated the mouse EDTA plasma or rat EDTA plasma samples were NOT detected by this ELISA Kit due the sample recovery of EDTA plasma samples is very low.

Research Samples Test:

The research mouse or rat serum pooled samples were diluted by Dilution Buffer DB01. The linearity and recovery was assayed by Mouse Rat ENPP2 ELISA Kit SK00526-01.

Sample	Dilution	Assayed (ng/mL)	Final (ng/mL)
Mouse Serum	2 X	7.258	14.516
Mouse Serum	4 X	3.867	15.468
Rat Serum	2 X	2.870	5.741
Rat Serum	4 X	1.715	6.860