LIPOCALIN 13 (MOUSE) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE LIPOCALIN 13 CONCENTRATIONS IN SERUM AND EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL 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	LIPOCALIN 13 (MOUSE) ELISA
Catalog No.	SK00648-01
Lot No.	
Formulation	96 T
Standard Range	312.5 – 20,000 pg/mL
Sensitivity	100 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Mouse Lipocalin-13at 100%; rat and fetal bovine serum samples indicated some cross-reaction
Calibration	Mouse Lipocalin 13 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Mouse Lipocalin 13 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Lipocalin 13 from mouse serum and EDTA plasma in a sandwich ELISA format.

This immunoassay contains recombinant mouse Lipocalin 13 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Lipocalin 13 in mouse samples. The data indicated rat serum or EDTA plasma and fetal bovine serum samples cross-react with this elisa. These results indicate that immunoassay of mouse lipocalin-13 in cell cultures should be used animal serum free culture media.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for mouse Lipocalin 13. The capture antibody can bind to the mouse Lipocalin 13 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against mouse Lipocalin 13 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 is added to the wells and color develops in direct proportion to the amount of mouse Lipocalin 13 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 with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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
Lipocalin 13 Microplate - 96 well polystyrene microplate coated with an antibody specific for mouse Lipocalin 13.	**648-01-01**	**1 plate**
Lipocalin 13 Standard – 20,000 pg/vial of lyophilized recombinant mouse Lipocalin 13.	**648-01-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of lyophilized biotinylated antibody against mouse Lipocalin 13.	**648-01-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant mouse Lipocalin 13.	**648-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µl of 100-fold concentrated solution of Streptavidi-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB09**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB26**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 10 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° 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) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Mouse serum and plasma samples may require a 25-fold dilution. A suggested 25-fold dilution is 10 µl = 240 µl Dilution Buffer.

Rat serum and plasma samples DO NOT require dilution. **Optimal dilutions should be determined by each laboratory for each application.**

Use polypropylene test tubes.

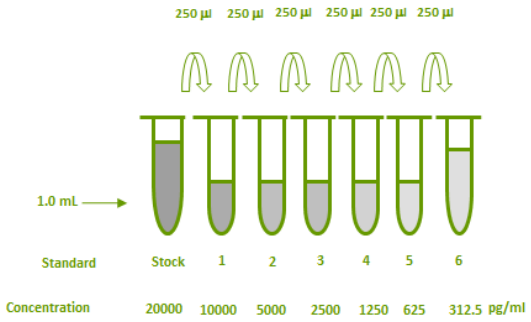
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Lipocalin 13 Standard - Reconstitute the Lipocalin 13 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 20,000 pg/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. The **20,000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	1000 µl	20,000 pg/ml
# 1	250 µl of stock	250 µl	10,000 pg/ml
# 2	250 µl of 1	250 µl	5000 pg/ml
# 3	250 µl of 2	250 µl	2500 pg/ml
# 4	250 µl of 3	250 µl	1250 pg/ml
# 5	250 µl of 4	250 µl	625 pg/ml
# 6	250 µl of 5	250 µl	312.5 pg/ml



Positive Control - Reconstitute the Positive Control with 2.0 mL of Dilution Buffer.

Detection Antibody – Reconstitute the Detection Antibody Concentrate with 1.2mL of **Antibody Diluent Solution (DB26)** to produce a 10-fold concentrated solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 9.45 mL of Antibody Diluent

Solution into a 15mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated solution to prepare 1x working solution.

Streptavidin-HRP Conjugate – Pipette 11.88 mL of **HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare 1x working solution. **Note:** 1x working solution of streptavidin-HRP should be used immediately **(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 and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL per well of Detection Antibody 1x working solution. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µl per well of 1x working solution of Streptavidin-HRP Conjugate. Cover with plate sealer and incubate for 40 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 8-10 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

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

Proteins	Cross-reactivity (%)
Mouse Lipocalin 13	100
Mouse Lipocalin 2	0
Rat Lipocalin 2	0
Human Lipocalin 2	0

Data also indicates that rat serum, rat EDTA plasma and fetal bovine serum binds to the antibody that was used in this kit formulation. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard, which means that rat or bovine samples cross-react with Mouse Lipocalin 13 ELISA Kit.

TYPICAL DATA

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

Standard (pg/mL)	Average OD450 (Corrected)
Blank	0 (0.111)
312.5	0.079
625	0.147
1250	0.259
2500	0.486
5000	0.787
10,000	1.107
20,000	1.422

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well 1x Detection Antibody working solution. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl per well of 1x Streptavidin-HRP conjugate working solution. Incubate 40 minutes on microplate shaker at RT. **Protect fom light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 8-10 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.