MOUSE SECRETED TWEAK ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE SECRETED TWEAK CONCENTRATIONS IN SERUM AND 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	**MOUSE sTWEAK ELISA KIT**
Catalog No.	SK00577-03A
Lot No.	
Formulation	96 T
Standard Range	62.5 – 4000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Sample Type	Serum and Plasma
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Mouse sTWEAK
Calibrate	Mouse secreted TWEAK recombinant
Intra-assay Precision	6 - 8%
Inter-assay Precision	10 - 12%
Storage	2 – 8° C for 1 month. See page 3 for detail
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Mouse Secreted TWEAK ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Mouse sTWEAK from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant Mouse secreted TWEAK and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural sTWEAK samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for Mouse sTWEAK. The capture antibody can bind to the Mouse sTWEAK in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against Mouse sTWEAK 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 sTWEAK 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.
_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
sTWEAK Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sTWEAK.	**577-03A-01**	**1 plate**
sTWEAK Standard – 4000 pg/vial of recombinant Mouse sTWEAK in a buffered protein base with preservatives; lyophilized.	**577-03A-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrated of biotinylated antibody against sTWEAK with preservatives; lyophilized.	**577-03A-03**	**1 vial**
Positive Control - one vial of recombinant Mouse sTWEAK, lyophilized (optional).	**577-03A-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer - 40 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB12**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB03**	**1 bottle**
TMB 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 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8°C for up to 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer, Antibody Diluent Solution and HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate should be stored 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

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.
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.

Optional: Use Aprotinin (enzyme inhibitor) (00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

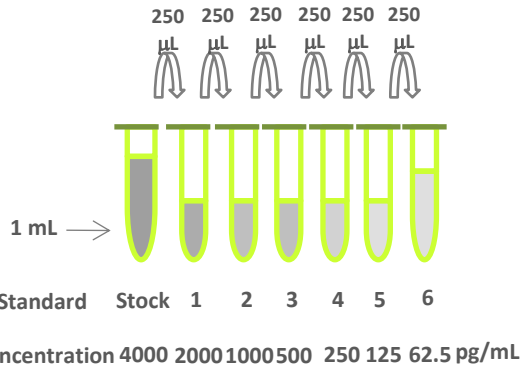
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Dilution Buffer (DB06) - Dilution Buffer (DB06) is highly viscous, warm in 30 - 37° C water bath until liquid flows more freely.

Antibody Diluent Solution (DB12) - Antibody Diluent Solution (DB12) is highly viscous, warm in 30 - 37° C water bath until liquid flows more freely.

sTWEAK Standard - Reconstitute the sTWEAK standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **4000 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	4000 pg/mL
# 1	250 µL of stock	250 µL	2000 pg/mL
# 2	250 µL of 1	250 µL	1000 pg/mL
# 3	250 µL of 2	250 µL	500 pg/mL
# 4	250 µL of 3	250 µL	250 pg/mL
# 5	250 µL of 4	250 µL	125 pg/mL
# 6	250 µL of 5	250 µL	62.5 pg/mL



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

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB12)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - 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**).

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. Add 100 µL per well of **Dilution Buffer** to Blank wells (B).
3. Add 100 µL of **Standard dilutions (6 to S)**, **samples**, or **positive control** (P) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
4. 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.
5. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for 20-25 minutes on micro-plate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 minutes.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log or 4-parameter curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the sTWEAK concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL STANDARD CURVE

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.095)
62.5	0.054
125	0.092
250	0.179
500	0.319
1000	0.545
2000	1.108
4000	2.135

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Mouse sTWEAK	100
Human sTWEAK	5
Mouse TNF-alpha	0

The rat serum or EDTA plasma samples showed highly cross-reactive with this mouse secreted ELISA Kit.

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 Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 20-25 minutes on the plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read at 450nm within 3 min.

Sample Test

The research polled mouse serum or EDTA plasma samples were diluted by Dilution Buffer DB06. It was detected by Mouse Secreted TWEAK ELISA Kit SK00577-03A.

Sample Type	Dilution Factor	Assayed (pg/mL)	Final (pg/mL)	Recovery (%)
Serum	1 X	309.933	309.933	100
Serum	2 X	155.964	311.928	99.4
Plasma	1 X	126.025	126.025	100
Plasma	2 X	57.346	114.692	91