HUMAN MIDKINE ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN MIDKINE CONCENTRATIONS IN SERUM, PLASMA AND CELL CULTURE SUPERNATES

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:

ELISA NAME	HUMAN MIDKINE ELISA KIT
Catalog No.	SK00153-01
Lot No.:	
Formulation	96 T
Standard range	31.2 - 2000 pg/mL
Sensitivity	15.6 pg/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA plasma and Cell Culture Supernates
Specificity	Human Midkine
Calibration	Human Midkine recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
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 Human Midkine ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Midkine from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Midkine and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Midkine samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Midkine. The capture antibody can bind to the human Midkine in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Midkine 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 human Midkine 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
Midkine Microplate – 96 well microplate coated with an antibody specific for human Midkine.	**153-01-01**	**1 plate**
Midkine Standard – 2000 pg/vial of lyophilized recombinant human Midkine.	**153-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial of 10-fold concentrate of lyophilized biotinylated antibody against human Midkine.	**153-01-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant human Midkine.	**153-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µl/vial of 200-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-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 0.5M 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 6 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.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detction Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution and other

components may be stored at 2 – 8° C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C.

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

Cell Culture Supernates - Remove particulates by centrifugation and 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.
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

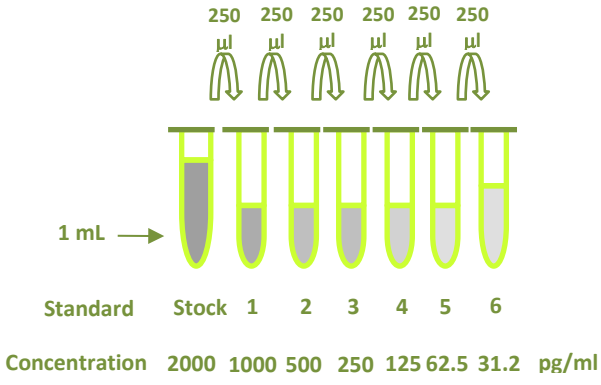
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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.

Midkine Standard - Reconstitute the Midkine standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 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 **2000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

STANDARD TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1 ml	2000 pg/ml
# 1	250µl of stock	250µl	1000 pg/ml
# 2	250µl of 1	250µl	500 pg/ml
# 3	250µl of 2	250µl	250 pg/ml
# 4	250µl of 3	250µl	125 pg/ml
# 5	250µl of 4	250µl	62.5 pg/ml
# 6	250µl of 5	250µl	31.25 pg/ml



Positive Control - Reconstitute the Positive Control with 2.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C ~ -70° C.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of

Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 9.45 mL of Dilution Buffer in a 15mL centrifuge tube to prepare Detection Antibody working solution.

Streptavidin-HRP Conjugate - Transfer 60 µl of 200-fold concentrated stock solution to 11.94 mL of **HRP Diluent Solution (DB08)** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(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 per well of Dilution Buffer to blank wells.
4. Add 100 µl per well of standard dilutions from #6 to #S (reverse order of serial dilution) to the wells. Add 100 µl per well of Positive control into wells. Add 100 µl per well of samples into appropriate wells. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of Detection Antibody working solution. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution. Cover with plate sealer and incubate at room temperature for 60 minutes on microplate shaker. **Protect from light**.
11. Repeat the aspiration/wash as in step 5.
12. Add 100 µL of Substrate Solution to each well. Incubate for 5-20 minutes at room temperature on microplate shaker. **Protect from light**.
13. 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
Human Midkine	100
Human PTN/OSF-1	<1
Human Neurturin	0
Human BDNF	0
Human NGF-beta	0
Human NT3	0
Human Persephin	0

TYPICAL DATA

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 (0.158)
31.25	0.024
62.5	0.051
125	0.095
250	0.147
500	0.260
1000	0.460
2000	1.032

- **Lot No.:**
- **Positive Control:**

LINEARITY

To assess the linearity of the assay, pooled research human serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
1X	516.460	516.460	100
2X	218.596	437.192	85

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl 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 all wells. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 5-20 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.