HUMAN MIDKINE ELISA KIT

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

PURCHASE 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	100ul
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
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	4 °C

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

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INTRODUCTION

Human Midkine immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human Midkine in serum, EDTA plasma and cell culture supernates. It contains recombinant human Midkine and antibodies raised against this protein. It has been shown to accurately quantify recombinant human Midkine. Results obtained with naturally occurring Midkine samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human Midkine.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Midkine has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any midkine present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for midkine is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of midkine bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute 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.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
MIDKINE Microplate – 96 well microplate precoated with an antibody against human Midkine	153-01-01	1 plate
MIDKINE Standard – 2 ng/vial of recombinant human Midkine in a buffered protein base with preservatives; lyophilized.	153-01-02	1 vial
MIDKINE Antibody Concentrate – 105µl / vial, 100-fold concentrated of antibody against human Midkine with preservatives; lyophilized.	153-01-03	1 vial
Positive Control – one vial of recombinant human Midkine, lyophilized (optional)	153-01-04	1 vial
Streptavidin-HRP Conjugate - 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer - 60 mL of buffered protein based solution with preservatives	DB01	1 bottle
Wash Buffer - 50 ml of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB Substrate Solution - 11ml of TMB substrate solution	TMB01	1 bottle
Stop Solution（0.5M HCl）, 11 ml of 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Antibody Concentrated should be stored at -20 or -70 °C. Do not use past kit expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Positive Control and Antibody SHOULD BE STORED at -20 °C or – 70°C for up to one month.

Streptavidin - HRP Conjugate 200-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch containing the desiccant pack, reseal along the entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

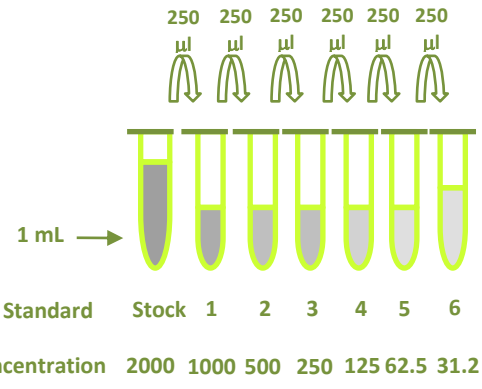
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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 - Refer to vial label for reconstitution volume. 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 the appropriate Dilution Buffer into the tube #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



MIDKINE Antibody Concentrate - Reconstitute the Antibody Concentrate with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Transfer it to 10.395 mL of Dilution Buffer to prepare 1X Antibody working solution.

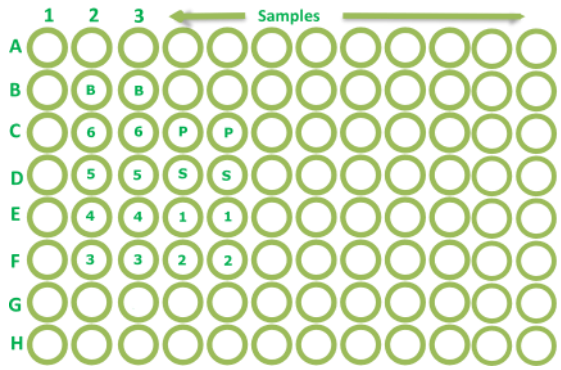
Streptavidin-HRP Conjugate - Transfer 60 µl of 200-fold concentrated stock solution to 11.94 ml of Dilution Buffer to prepare working solution. **Note:** 1X working solution of Streptavidin HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 2.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Leave well B2 and B3 as Blank. Add 100 µl per well of Dilution Buffer.
4. Add 100 µl per well of standard solution from #6 to #S (reverse order of serial dilution) to the appropriate wells (C2, C3 to F2, F3 and F4, F5 to D4, D5). Add 100 µl per well of Positive control into wells C4 and C5. Add 100 µl per well of samples into appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm). **Note:** Standard, Blank and PC should be assayed in duplicates.
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 1X Antibody working solution. Cover or seal the plate 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 or seal the plate 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 8-12 minutes at room temperature. **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 micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a log-log curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Midkine.

SENSITIVITY

The minimum detectable dose (MDD) of human Midkine was 15.6 pg/mL.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated 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: 700-1200 pg/ml**

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

SPECIFICITY

This assay recognizes both natural and recombinant human Midkine. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

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

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl 1X 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 8-12 min on the bench top. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min