HUMAN CARDIOTROPHIN-LIKE CYTOKINE FACTOR 1 (CLCF1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CLCF1 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

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	**HUMAN CLCF1 ELISA**
Catalog No.	SK000158-06
Formulation	96 T
Lot No.	
Standard range	125 ~ 8000 pg/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, EDTA Plasma,
Specificity	Human CLCF1
Calibration	Human CLCF1 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C for 1 month. See page 2-3 for detail
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human CLCF1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CLCF1 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CLCF1 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CLCF1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human CLCF1. The capture antibody can bind to the human CLCF1 in the standard and samples. After washing the plate of any unbound substances, the biotinylated monoclonal antibody against human CLCF1 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 CLCF1 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
CLCF1 Microplate – 96 well microplate coated with a monoclonal antibody specific for human CLCF1.	**158-06-01**	**1 plate**
CLCF1 Standard – 8 ng per vial of lyophilized recombinant human CLCF1.	**158-06-02**	**1 vial**
Detection Antibody Concentrate – 1.2mL of 10-fold concentrate of liquid antibody against human CLCF1.	**158-06-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant human CLCF1.	**158-06-04**	**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.	**DB06**	**1 bottle**
Antibody and HRP Diluent Solution – 25 mL of buffered solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer – 25 mL of 20-fold concentrated buffered surfactant with preservative.	**WB02**	**1 bottle**
TMB Substrate Solution – 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.125M 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 8 months. For longer storage for up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & 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.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store 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 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.

Dilution Buffer (DB06) - Dilution Buffer (DB03) is highly viscous, warm in 30 - 37° C water bath until liquid flows more freely.

CLCF1 Standard – Reconstitute the CLCF1 standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 pg/mL . Mix each tube thoroughly before the next transfer. Create a standard curve using a 2-fold serial dilution in Dilution Buffer with a high standard of **8 ng/mL** is recommended.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1 mL	8000 pg/mL
# 1	250 µL of stock	250 µL	4000 pg/mL
# 2	250 µL of 1	250 µL	2000 pg/mL
# 3	250 µL of 2	250 µL	1000 pg/mL
# 4	250 µL of 3	250 µL	500 pg/mL
# 5	250 µL of 4	250 µL	250 pg/mL
# 6	250 µL of 5	250 µL	125 pg/mL

Positive Control - Reconstitute the Positive Control with refer to specific lot of Dilution Buffer to produce working solution.

Detection Antibody - Pipette 9.45 mL of **Antibody and HRP Diluent Solution (DB08C)** 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 9.9 mL of **Antibody and HRP Diluent Solution (DB08C)** into a 15 mL centrifuge tube and transfer 100 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**, **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 90 min 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 45 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 15-20 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

Protein	Cross-reactivity (%)
Human CLCF1	100
Human IL-6	0
Human CNTF	0
Human ST2	0
Human GDF15	0
Human FSTL1	0

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 NM (CORRECTED)
Blank	0 (0.089)
125	0.032
250	0.066
500	0.129
1000	0.269
2000	0.541
4000	1.132
8000	2.227

Lot No.:

Positive Control : refer to lot

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL of standard dilutions, samples or 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 min 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 15-20 on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 3 min.