HUMAN CCL14 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CCL14 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	CCL14 (HUMAN) ELISA KIT
Catalog No.	SK00446-06
Lot No.	
Formulation	96 T
Standard range	15.6 – 1000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma
Dilution Factor	***For serum or plasma : 10~20 (Optimal dilutions should be determined by each laboratory for each application)***
Specificity	Human CCL14
Calibration	Human CCL14 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C for 1 month, see page 2-3 for more information
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 Human CCL-14 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural CCL14 from serum samples and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CCL14 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human CCL14 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with a monoclonal antibody specific for human CCL14. The capture antibody can bind to the human CCL14 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human CCL14 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 human CCL14 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.
_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
CCL14 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human CCL14.	**446-06-01**	**1 plate**
Human CCL14 Standard – 1000 pg/vial of recombinant human CCL14 in a buffered protein base with preservative; lyophilized.	**446-06-02**	**1 vial**
Detection Antibody Concentrate – 1.05 ml/vial, 10-fold concentrated of biotinylated antibody solution against human CCL14 with preservative	**401-01-03**	**1 vial**
Positive Control –one vial, of human CCL14 recombinant with preservative	**446-06-04**	**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 & HRP Diluent Solution – 25 mL of buffered protein based solution with preservative.	**DB16**	**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 solution.	**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 up to 10 months, unopened Standard, 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 PREPARATION

Serum or plasma samples may require 10 ~20 fold dilution.

A suggested 10-fold dilution is 25 µL sample + 225 µL Dilution Buffer. A suggested 20-fold dilution is 12.5 µL sample + 237.5 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application with a sample 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 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.**

CCL14 Standard - Reconstitute the CCL14 standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 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 the tube #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1000 pg/ml
# 1	250 µl of stock	250 µl	500 pg/ml
# 2	250µl of 1	250µl	250 pg/ml
# 3	250µl of 2	250µl	125 pg/ml
# 4	250µl of 3	250µl	62.5 pg/ml
# 5	250µl of 4	250µl	31.25 pg/ml
# 6	250µl of 5	250µl	15.6 pg/ml

Positive Control - Reconstitute the Positive Control with 1 mL Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Antibody & HRP Diluent Solution (DB16) to prepare 10-fold concentrated stock. Pipette 9.45 mL of Antibody & HRP Diluent Solution (DB16) 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 10.89 mL of Antibody & HRP Diluent Solution (DB16) into a 15 mL centrifuge tube and transfer 110µL 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.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate 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 microplate 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 microplate 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 10-20 minutes on microplate 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 min.

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 4-parameter 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 CCL14	100
Human CCL18	0

TYPICAL STANDARD CURVE

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 (0.101)
15.6	0.042
31.25	0.083
62.5	0.156
125	0.322
250	0.629
500	1.219
1000	2.394

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the 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 min on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate 10-20 min on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read at 450 nm within 3 min.