

CHEMOKINE CCL17/TRAC ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF
HUMAN CCL17 CONCENTRATIONS IN CELL CULTURE
SUPERNATES AND SERUM



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 CCL17/TRAC ELISA KIT
Catalog No.	SK00467-01
Lot No.	
Formulation	96 T
Standard range	15.6 - 1000 pg/mL
Sensitivity	3 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma Cell Culture Supernates
Dilution Factor	<i>Optimal dilutions should be determined by each laboratory for each application</i>
Specificity	Human CCL17
Calibration	Human CCL17 Rec
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 CCL17/TRAC ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural CCL17/TRAC from serum samples and cell culture supernates in a sandwich ELISA format.

This immunoassay contains recombinant CCL17/TRAC derived from CHO and antibodies raised against CCL17/TRAC recombinant derived from CHO. Results from this immunoassay have shown to accurately quantify recombinant and natural active CCL17/TRAC samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich enzyme immunoassay technique. The plate is pre-coated with an antibody specific for CCL17/TRAC. The capture antibody can bind to the CCL17/TRAC in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against CCL17/TRAC 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 CCL17/TRAC 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
CCL17 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against CCL17.	467-01-01	1 plate
CCL17 Standard – 1000 pg/vial of recombinant CCL17 in a buffered protein base with preservative; lyophilized.	467-01-02	1 vial
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrated of biotinylated antibody against CCL17 with preservative; lyophilized.	467-01-03	1 vial
Positive Control - one vial of recombinant CCL17; lyophilized. (optional)	467-01-04	1 vial
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB01	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.	WB01	1 bottle
TMB Substrate Solution - 11 mL of TMB substrate solution.	TMB01	1 bottle
Stop Solution - 11 mL of 0.25M 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 for up to 12 months, unopened

Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C or -70° 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 (350 – 450 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. Please use animal free media for cell cultures samples assay.

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

Serum or Plasma samples may require a 2-4 fold dilution.

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.

CCL17 Standard - Reconstitute the CCL17 standard with 1.0 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 #1 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 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	1000 pg/ml
# 1	250 µl of stock	250µl	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 (optional) - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of HRP Diluent solution 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.
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 10-15 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 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

PROTEINS	CROSS-REACTIVITY (%)
Human CCL17	100
Human CCL18	0
Human CXCL9	0
Human CXCL10	0
Human IL-6	0
Human IL-1 beta	0
Human IL-4	0
Human TNF-alpha	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.

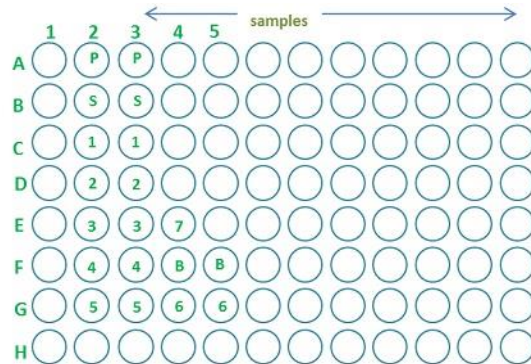
STANDARD (PG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.088)
15.6	0.049
31.25	0.110
62.5	0.215
125	0.425
250	0.877
500	1.676
1000	2.652

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 10-15 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.

The research pooled samples were assayed by Human CCL17/TRAC ELISA Kit SK00467-01

Sample	Dilution Factors	Assayed (pg/ml)	Final (pg/ml)	Recovery (%)
Human Serum	2 X	624.019	1248.038	100
Human Serum	4 X	311.073	1244.292	99.7
Human Plasma	2 X	511.036	1022.072	100
Human Plasma	4 X	249.118	996.472	97.5



Human CCL17 / TRAC ELISA Kit SK00467-01

Standard Range: 15.6 - 1000 pg/mL

Sensitivity: 3 pg/mL

Specificity: Human CCL17

