Quantikine™ ELISA

Human CCL27/CTACK Immunoassay

Catalog Number DCC270

For the quantitative determination of human Cutaneous T Cell Attracting Chemokine (CTACK) concentrations in cell culture supernates, serum and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Cutaneous T Cell-Attracting Chemokine (CTACK), also known as CCL27, is a CC chemokine specific for cells expressing CC chemokine receptor-10 (CCR10) and was discovered through the screening of a skin cell derived EST database and has also been reported as ILC, ALP, and ESkine (1-4). CTACK expression is confined mainly to the skin where it attracts memory T lymphocytes featuring cutaneous lymphocyte-associated antigen (CLA) (1, 5, 6). Cutaneous inflammatory conditions involving these skin-homing T cells include atopic dermatitis, allergic contact dermatitis, and psoriasis (6).

Human CTACK cDNA encodes a precursor protein of 112 amino acids (aa) with a 24 aa signal sequence that is cleaved to yield the 88 aa mature factor (1). An mRNA splice variant of CTACK that may or may not be targeted to the nucleus has also been reported (3), however, the significance of this splice variant is unknown. Mouse CTACK is 84% similar to the mature human protein in primary structure (1). The human CTACK gene has been mapped to chromosome 9 (1).

The CTACK receptor CCR10 is a seven-transmembrane G-protein coupled receptor originally identified as orphan receptor GPR-2 (7, 8). CCR10 expression has been detected on the surface of CLA^+ T lymphocytes, dermal microvascular endothelial cells, and dermal fibroblasts (6), but mRNA expression studies suggest it is present on many other tissues and cells (7-11). Other factors capable of binding CCR10 include CCL28 (12, 13) and the broad-spectrum CC chemokine receptor antagonist vMIP-II from human herpes virus-8 (14).

Although CCR10 mRNA expression has been observed in many tissues and cell types, CTACK is expressed almost exclusively within skin. Both normal and diseased skin types constitutively express CTACK (1, 6). *In vitro* expression can be induced beyond constitutive levels by stimulation with IL-1β or TNF-α (1, 6), while gluticosteroids can suppress it (6). CTACK has been shown to predominantly attract skin-specific CLA^+ memory T lymphocytes. CLA is the ligand for E-selectin (15), an adhesion molecule that is critical in recruiting T cells to sites of cutaneous inflammation (16). In healthy mice, lymphocytes accumulate at sites of CTACK injection (6). In a mouse contact-hypersensitivity model, lymphocyte recruitment is impaired by administration of anti-CTACK neutralizing antibodies (6). The body of evidence accumulated to date indicates that normal skin-associated immuno-surveillance, as well as healthy and pathological cutaneous inflammatory responses, are influenced by the CTACK/CCR10 interaction. For a review on the tissue-specific migration of lymphocytes, see reference 17.

The Quantikine™ Human CCL27/CTACK Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human CTACK in cell culture supernates, serum and plasma. It contains *E. coli*-expressed recombinant human CTACK and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human CTACK showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human CTACK.

PRINCIPLE OF THE ASSAY

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

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human CTACK Microplate	892161	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human CTACK.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human CTACK Conjugate	892162	21 mL of a monoclonal antibody specific for human CTACK conjugated to horseradish peroxidase, with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human CTACK Standard	892163	Recombinant human CTACK in a buffered protein base with preservative, lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1S	895137	11 mL of a buffered protein base with preservative.	
Calibrator Diluent RD6-21	895261	21 mL of a buffered animal serum with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 500 mL graduated cylinder
- **Polypropylene** test tubes for dilution of standards
- Human CTACK Controls (optional; R&D Systems®, Catalog # QC71)

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 at room temperature 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 or heparin 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: *Citrate plasma has not been validated for use in this assay.*

REAGENT PREPARATION

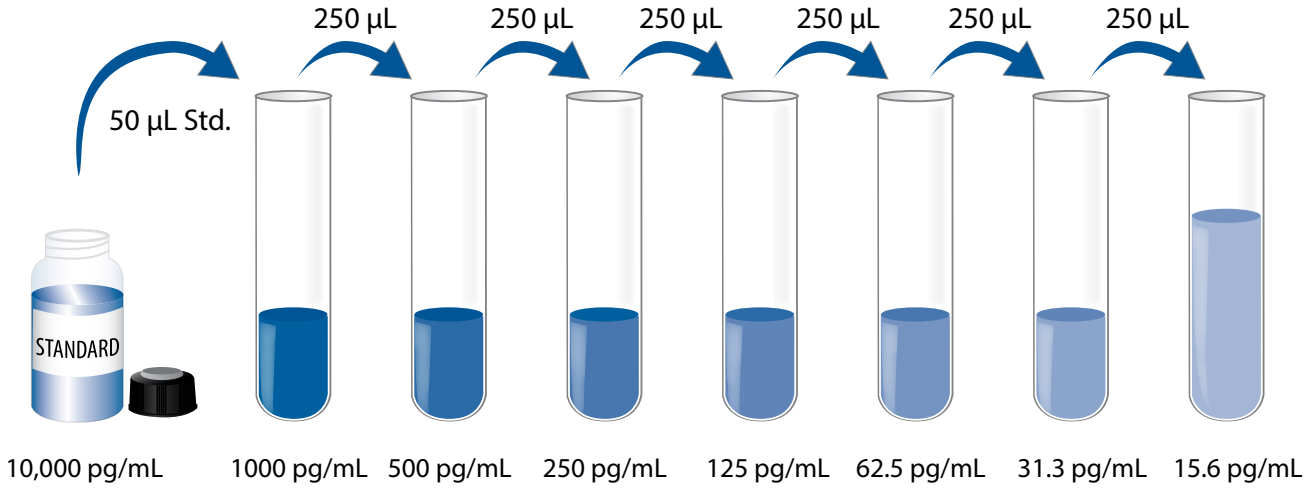
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human CTACK Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CTACK Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 450 µL of Calibrator Diluent RD6-21 into the 1000 pg/mL tube. Pipette 250 µL into the remaining tubes. 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. Calibrator Diluent RD6-21 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Assay Diluent RD1S to each well.
4. Add 50 µL of standard, control or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 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.
6. Add 200 µL of Human CTACK Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

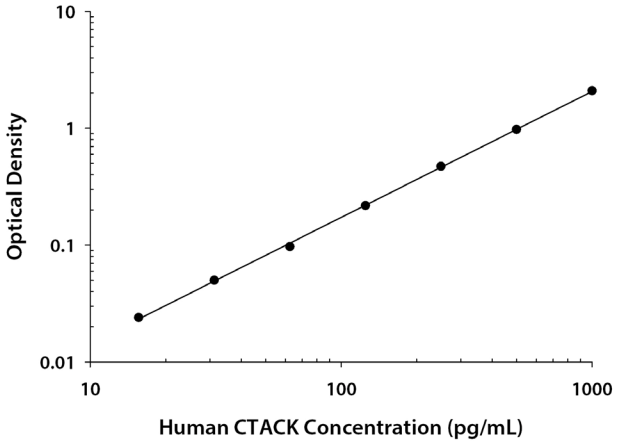
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human CTACK concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.008 0.009	0.008	—
15.6	0.031 0.034	0.032	0.024
31.3	0.057 0.059	0.058	0.050
62.5	0.103 0.107	0.105	0.097
125	0.225 0.228	0.226	0.218
250	0.479 0.480	0.480	0.472
500	0.961 1.006	0.984	0.976
1000	2.020 2.181	2.100	2.092

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	127	241	559	129	242	557
Standard deviation	4.79	9.05	28.4	16.1	21.5	33.8
CV (%)	3.8	3.8	5.1	12.5	8.9	6.1

RECOVERY

The recovery of human CTACK spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	91-105%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human CTACK were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	104	106	104	104
	Range (%)	103-105	104-109	99-107	93-110
1:4	Average % of Expected	108	106	106	107
	Range (%)	103-112	104-108	97-113	103-114
1:8	Average % of Expected	107	104	105	104
	Range (%)	100-111	101-107	99-110	94-114
1:16	Average % of Expected	106	100	101	101
	Range (%)	97-112	96-105	89-108	94-113

SENSITIVITY

Forty-five assays were evaluated and the minimum detectable dose (MDD) of human CTACK ranged from 0.50-4.68 pg/mL. The mean MDD was 1.55 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human CTACK produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human CTACK in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=50)	522	266-788	125
EDTA plasma (n=35)	503	272-685	103
Heparin plasma (n=35)	484	257-702	106

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernates were removed and assayed for levels of human CTACK. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human CTACK.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human CTACK control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

6Ckine
Eotaxin
Eotaxin-2
Eotaxin-3 (aa 27-94)
Eotaxin-3 (aa 24-94)
HCC-1
HCC-4
I-309
MCP-1
MCP-2
MCP-3
MCP-4
MDC
MIP-1α (66 aa)
MIP-1α (70 aa)
MIP-1β
MIP-1δ (68 aa)
MIP-1δ (92 aa)
MIP-3α
MIP-3β
MPIF-1
PARC
RANTES
TARC
TECK

Recombinant mouse:

6Ckine
CTACK
Eotaxin
JE/MCP-1
MARC
MCP-5
MDC
MIP-1α
MIP-1β
MIP-1γ
MIP-3α
MIP-3β
RANTES
TARC
TECK
TCA-3

Recombinant rat:

MIP-3α

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