Human CCL28 Immunoassay

Catalog Number: EA800055

For the quantitative determination of human CCL28 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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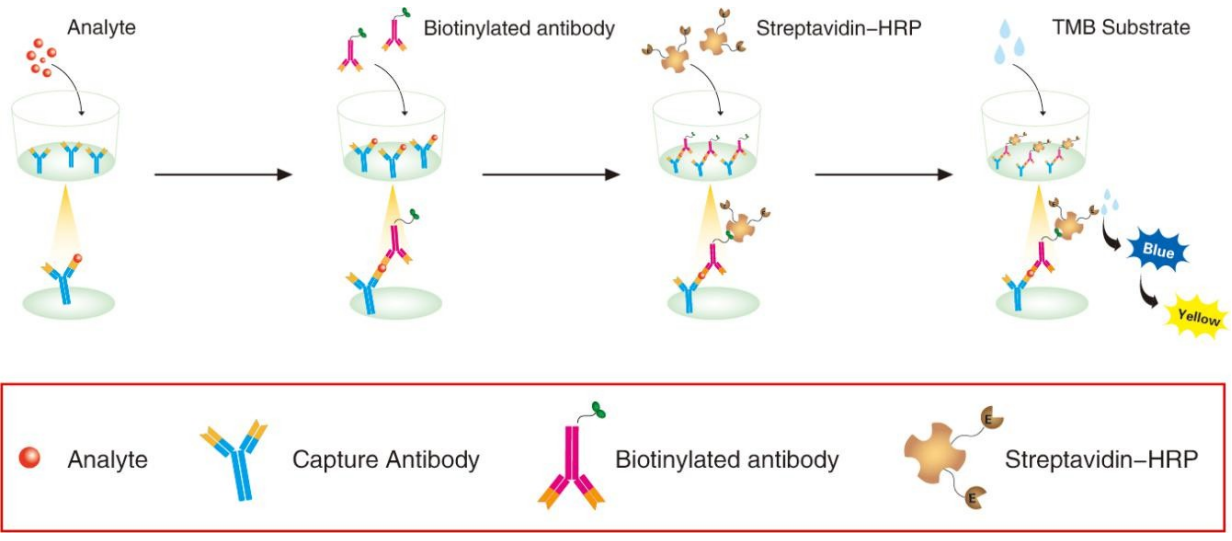
BACKGROUND

Human CCL28 (CC chemokine ligand 28) is a novel CC chemokine identified by TBLASTN searches of the Human Genome Systems (HGS) and Genbank dbEst database using a human chemokine consensus sequence. Human CCL28 cDNA encodes a 127 amino acid (aa) residue precursor protein with a putative 22 aa residue signal peptide that is cleaved to produce the 105 aa residue mature protein. Human and mouse CCL28 are highly conserved, sharing 83% aa identity in their mature regions. Among CC chemokines, CCL28 shares the most homology with CCL27/CTACK. The mouse CCL28 gene has been mapped to the distal region of chromosome 13. Human and mouse CCL28 RNA expression was found to be highest in normal and pathologic colon with the protein being expressed by epithelial cells. Human CCL28 RNA was also present in normal and asthmatic lung tissues. The receptor for CCL28 has been identified as the CCR10 (GPR2 orphan receptor) which is also the receptor for CCL27/CTACK.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CCL28 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CCL28 present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for CCL28 is added to detect the captured CCL28 protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



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1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	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℃**
Standard - lyophilized,8000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard/Sample Diluent - 16 ml/ vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

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

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

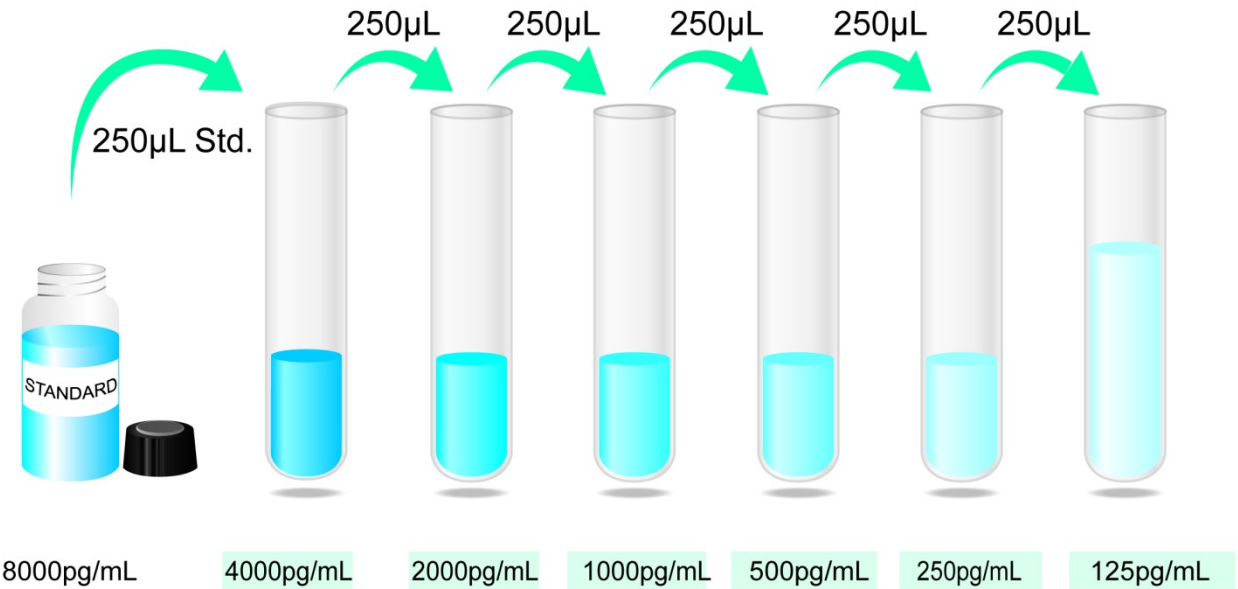
Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. 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×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.

2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard/Specimen** - Reconstitute the Standard with 0.5mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 8000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Standard/Sample Diluent into 4000pg/ml tube and the remaining tubes. Use the stock solution of 8000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 8000 pg/mL standard serves as the high standard. The



Preparation of CCL28 standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-human CCL28 antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

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Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human CCL28 antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

Aspirate and wash 4 times

Add 100µl working solution of Streptavidin-HRP to each well, shaking with Micro-oscillator (100r/min) to incubate 30 minutes at room temperature(25±2℃).

Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-20 minutes (depending on signal),at room temperature(25±2℃).Protect from light.

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

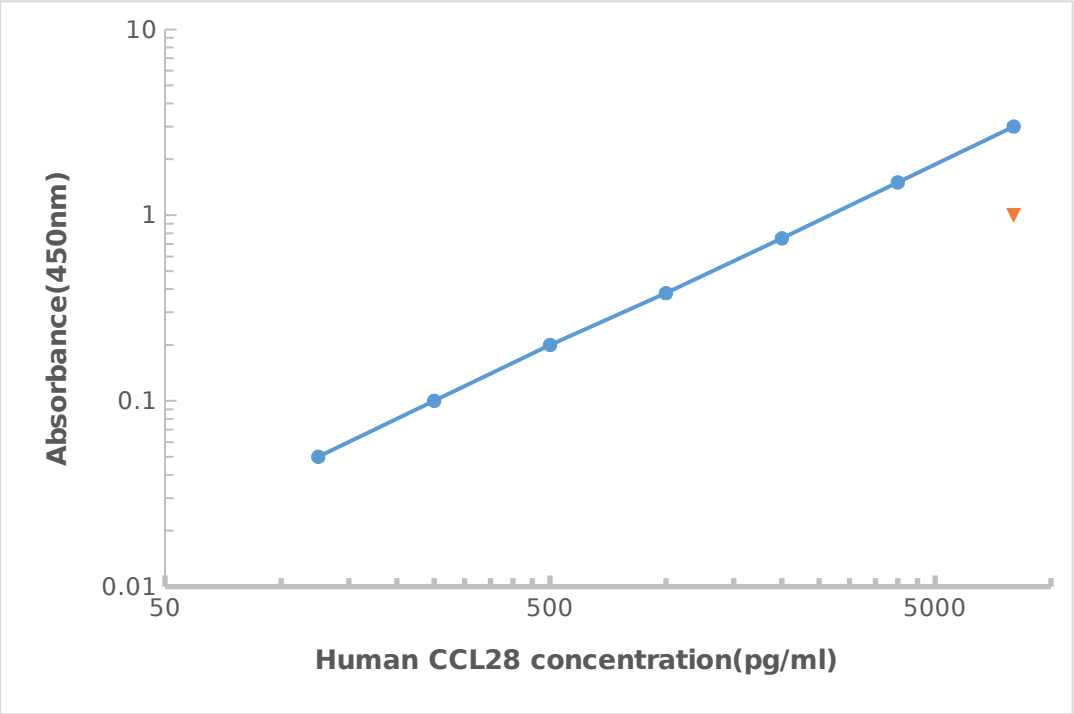
CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph.
4. The data may be linearized by plotting the log of the CCL28 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the CCL28 ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0	**0.082**	**0.083**	**0.0825**	---
125	**0.156**	**0.162**	**0.159**	**0.0765**

250	**0.273**	**0.258**	**0.2655**	**0.183**
500	**0.419**	**0.441**	**0.43**	**0.3475**
1000	**0.662**	**0.635**	**0.6485**	**0.566**
2000	**1.018**	**1.046**	**1.032**	**0.9495**
4000	**1.865**	**1.846**	**1.8555**	**1.773**
8000	**2.809**	**2.847**	**2.828**	**2.7455**



Representative standard curve for CCL28 ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 60pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human CCL28. The factors

listed below were prepared at 100ng/ml in Standard /Sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
CTACK	CCL28	

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of CCL28 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of CCL28 in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	91	83-101
Cell culture supernatants	93	84-103

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of CCL28 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	94	105
	Range (%)	86-102	96-114
1:4	Average% of Expected	96	103
	Range (%)	88-104	95-111
1:8	Average% of Expected	95	104
	Range (%)	84-106	95-113
1:16	Average% of Expected	98	106
	Range (%)	90-110	98-113

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