

## Human CCL21/6Ckine/Exodus 2 ELISA Kit

**Catalog Number:** EA100382

### Assay Principle

The OriGene Human CCL21 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human CCL21 with a 96-well strip plate that is pre-coated with antibody specific for CCL21. The detection antibody is a biotinylated antibody specific for CCL21. The capture antibody is a monoclonal antibody from mouse, the detection antibody is a biotinylated polyclonal antibody from goat. The kit contains recombinant Human CCL21 with immunogen: Expression system for standard: E.coli, Immunogen sequence: S24-P134. The kit is analytically validated with ready to use reagents.

To measure Human CCL21, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Human CCL21 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human CCL21 in the sample.

### Overview

<b>Product Name</b>	Human CCL21/6Ckine/Exodus 2 ELISA Kit
<b>Reactive Species</b>	Human
<b>Size</b>	96wells/kit, with removable strips.
<b>Description</b>	Sandwich High Sensitivity ELISA kit for Quantitative Detection of human CCL21. 96wells/kit, with removable strips.
<b>Sensitivity</b>	<12 pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
<b>Detection Range</b>	62.5 pg/ml- 4000 pg/ml
<b>Storage Instructions</b>	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
<b>Uniprot ID</b>	O00585

## Technical Details

<b>Capture/Detection Antibodies</b>	The capture antibody is a monoclonal antibody from mouse, the detection antibody is a biotinylated polyclonal antibody from goat.
<b>Specificity</b>	Natural and recombinant Human CCL21
<b>Immunogen</b>	Expression system for standard: <i>E.coli</i> , Immunogen sequence: S24-P134
<b>Cross Reactivity</b>	There is no detectable cross-reactivity with other relevant proteins.

## Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

## Kit Components/Materials Provided

Description	Quantity	Volume	Storage of opened/reconstituted material
<b>Anti-Human CCL21 Pre-coated 96-well strip microplate</b>	1	12 strips of 8 wells	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
<b>Human CCL21 Standard</b>	2	10ng/tube	Discard the stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.
<b>Human CCL21 Biotinylated antibody (100x)</b>	1	100 µl	May be stored for up to 1 month at 4°C provided this is within the expiration date of the
<b>Avidin-Biotin-Peroxidase Complex (100x)</b>	1	100 µl	
<b>Sample Diluent</b>	1	30ml	
<b>Antibody Diluent</b>	1	12ml	
<b>Avidin-Biotin-Peroxidase Diluent</b>	1	12ml	
<b>Color Developing Reagent (TMB)</b>	1	10ml	
<b>Stop Solution</b>	1	10ml	
<b>Wash Buffer (25 x)</b>	1	20 ml	
<b>Plate Sealers</b>	4	Piece	

## Required Materials That Are Not Supplied

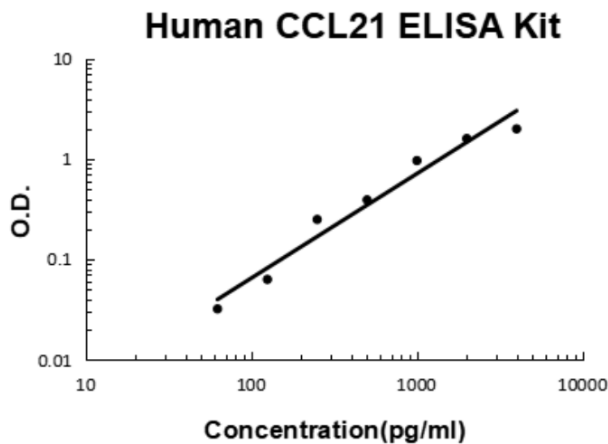
- Microplate Reader capable of reading absorbance at 450nm.
- Automated plate washer (optional)
- Pipettes and pipette tips capable of precisely dispensing 0.5  $\mu$ l through 1 ml volumes of aqueous solutions.
- Multichannel pipettes are recommended for large amount of samples.
- Deionized or distilled water.
- 500ml graduated cylinders.
- Test tubes for dilution.

## Human CCL21 ELISA Kit (EA100382) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	62.5	125	250	500	1000	2000	4000
O.D.	0.004	0.036	0.067	0.254	0.396	0.970	1.607	2.007

**Human CCL21 ELISA Kit Standard Curve**



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

## Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

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

**Inter-Assay Precision (Precision across assays):** Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	100	709	1352	103	706	1475
Standard deviation	4	28.36	82.47	6.9	31.77	92.92
CV(%)	4 %	4 %	6.1%	6.7 %	4.5 %	6.3 %

## Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	100	109	108	95	103	5.78	5.6%
Sample 2	709	697	706	724	709	9.72	1.3%
Sample 3	1352	1517	1447	1423	1434	58.95	4.1%

\*number of samples for each test n=16.

## Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-20min) is based on 37°C.
Wash buffer	Prepare 500 ml of working Wash Buffer by diluting the suspended 20 ml Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-Human CCL21 antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human CCL21 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human CCL21 Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the

	<p>experiment. Use one 10ng of lyophilized Human CCL21 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.</p>
Microplate	<p>The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.</p>
Samples	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Internal QC testing used dilution ratio of 1:1; concentration in serum was around 500 pg/ml.</p>

## Dilution of Human CCL21 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 – 4000pg/ml, #2 – 2000pg/ml, #3 – 1000 pg/ml, #4 – 500 pg/ml, #5 – 250 pg/ml, #6 – 125 pg/ml, #7 – 62.5 pg/ml, #8 – 0.0 (Blank).
2. To generate standard #1, add 400µl of the reconstituted standard stock solution of 10 ng/ml and 600µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

## Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.
Cell lysate	Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10,000 x g for 5 min. Collect the supernatant, assay immediately or store samples at -20°C.

## Sample Collection Notes

1. It is recommended that samples are immediately used upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25 °C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

## Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

## Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Human CCL21 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
  - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
  - b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
  - c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
  - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
  - b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
  - c. Repeat steps a-b 4 additional times.

11. Add 90  $\mu$ l of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100  $\mu$ l of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

## Assay Protocol Notes

1. *Solutions:* To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
2. *Applying Solutions:* All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
3. *Assay Timing:* The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
4. *Incubation:* To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
5. *Washing:* Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
6. *Controlling Substrate Reaction Time:* After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. The excessively strong color will result in inaccurate absorbance readings.
7. *Reading:* The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
8. *Reaction Time Control:* Control reaction time should be strictly followed as outlined.
9. *Stop Solution:* The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.
10. To minimize the external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

## Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: [www.myassays.com/four-parameter-logistic-curve.assay](http://www.myassays.com/four-parameter-logistic-curve.assay).

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

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## Background on CCL21

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Chemokine (C-C motif) ligand 21 (CCL21) is a small cytokine belonging to the CC chemokine family. This chemokine is also known as 6CKine (because it has six conserved cysteine residues instead of the four cysteines typical to chemokines), exodus-2, and secondary lymphoid-tissue chemokine (SLC).<sup>1</sup> By somatic cell hybrid and radiation hybrid analyses, mapped the SCYA21 gene to 9p13. <sup>2</sup> Chemokines are a family of proteins that direct leukocyte migration and activation to inflammatory stimuli. CXC chemokine ligand 13 (CXCL13), CC chemokine ligand 21 (CCL21), and CCL19 are constitutively expressed in secondary lymphoid organs, where they control the placement of lymphocytes and dendritic cells. It was demonstrated that the local expression of homeostatic chemokines in nonlymphoid organs, such as the lung, plays an important role in protective immune responses.<sup>3</sup>

### Reference

1. Hedrick JA, Zlotnik A (1997). "Identification and characterization of a novel beta chemokine containing six conserved cysteines". *J. Immunol.* 159 (4): 1589-93.
2. Nagira, M., Imai, T., Hieshima, K., Kusuda, J., Ridanpaa, M., Takagi, S., Nishimura, M., Kakizaki, M., Nomiyama, H., Yoshie, O. Molecular cloning of a novel human CC chemokine secondary lymphoid-tissue chemokine that is a potent chemoattractant for lymphocytes and mapped to chromosome 9p13. *J. Biol. Chem.* 272: 19518-19524, 1997.
3. Rangel-Moreno, J., Moyron-Quiroz, J. E., Hartson, L., Kusser, K., Randall, T. D. Pulmonary expression of CXC chemokine ligand 13, CC chemokine ligand 19, and CC chemokine ligand 21 is essential for local immunity to influenza. *Proc. Nat. Acad. Sci.* 104: 10577-10582, 2007.