CXCL14 (Pig) ELISA Kit

Catalog Number KA5390

96 assays

Version: 01

Intended for research use only

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Introduction

Intended Use

For the quantitation of pig CXCL14 concentrations in cell culture supernates, serum and plasma (heparin, EDTA).

Background

Chemokine (C-X-C motif) ligand 14 (CXCL14) is a small cytokine belonging to the CXC chemokine family that is also known as BRAK (for breast and kidney-expressed chemokine). This gene is mapped to 5q31.1. CXCL14 is constitutively expressed at high levels in many normal tissues, it has been found that fibroblasts are the primary source of CXCL14 and that CXCL14 is involved in the homeostasis of monocyte-derived macrophages rather than in inflammation. It is also a potent chemoattractant and activator of dendritic cells, is implicated in homing of these cells, and can stimulate the migration of activated NK cells. CXCL14 also inhibits angiogenesis, possibly as a result of its ability to block endothelial cell chemotaxis. In addition to it, CXCL14 is a potent chemokine for immature DCs, with a chemoattractive activity comparable to bacterial fMLP and MIP1A.

Principle of the Assay

CXCL14 (Pig) ELISA Kit is a solid phase immunoassay specially designed to measure pig CXCL14 with a 96-well strip plate that is pre-coated with antibody specific for CXCL14. The detection antibody is a biotinylated antibody specific for CXCL14. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Pig CXCL14 with immunogen: Expression system for standard: E.coli; Immunogen sequence: S35-E111. The kit is analytically validated with ready to use reagents.

To measure Pig CXCL14, 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 propotional to Pig CXCL14 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 Pig CXCL14 in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Anti-Pig CXCL14 Pre-coated 96-well strip microplate	96 (8x12) wells
Pig CXCL14 Standard	10 ng/tube x 2
Pig CXCL14 Biotinylated antibody (100x)	130 µL
Avidin-Biotin-Peroxidase Complex (100x)	130 µL
Sample Diluent	30 mL
Antibody Diluent	12 mL
Avidin-Biotin-Peroxidase Diluent	12 mL
Color Developing Reagent (TMB)	10 mL
Stop Solution	10 mL
Plate Sealers	4 slides

Storage Instruction

Store at 4 °C for 6 months, at -20 °C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate Reader capable of reading absorbance at 450 nm.
- ✓ Automated plate washer (optional).
- ✓ Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- ✓ Multichannel pipettes are recommended for large amount of samples.
- ✓ Deionized or distilled water.
- ✓ 500 mL graduated cylinders.
- ✓ Test tubes for dilution.
- ✓ Washing buffer (neutral PBS or TBS).
- Preparation of 0.01 M TBS:

Add 1.2 g Tris, 8.5 g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to 1000 mL H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.

- Preparation of 0.01 M PBS:

Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 mL distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.

Precautions for Use

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

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.

Assay Protocol

Reagent Preparation

✓ 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 (20-25 min) is based on 37°C.

- Biotinylated Anti-Pig CXCL14 antibody

  It is recommended to prepare this reagent immediately prior to use by diluting the Pig CXCL14 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-BiotinPeroxidase 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.

- Pig CXCL14 Standard

  It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Pig CXCL14 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/mL using 1 mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

- Microplate

  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.

✓ Dilution of Pig CXCL14 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 – 4000 pg/mL, #2 – 2000 pg/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 400 µL of the reconstituted standard stock solution of 10 ng/mL and 600 µL of sample diluent to tube #1 for a final volume of 1000 µL. 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

✓ 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.

- 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.*

✓ Sample Dilution Guideline

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 Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Reagent Preparation 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 minutes 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-Pig CXCL14 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 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 µL of Color Developing Reagent to each well and incubate in the dark for 30 minutes at RT or 25-30 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 µL 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 450 nm

Data Analysis

Calculation of Results

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.

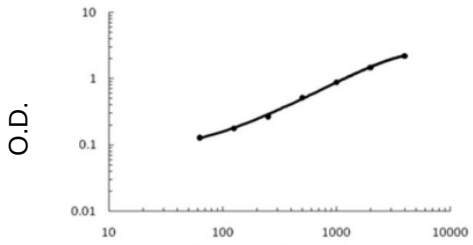
Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but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

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

✓ CXCL14 (Pig) ELISA Kit 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	0.012	0.018	0.053	0.097	0.240	0.724	1.268	2.184



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

Performance Characteristics

✓ Detection Range: 62.5 pg/mL-4000 pg/mL

✓ Sensitivity: < 10 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.

✓ Specificity: Natural and recombinant Pig CXCL14

✓ Cross-reactivity: There is no detectable cross-reactivity with other relevant proteins.

✓ Precision

- 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 accross assays). Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/mL)	987	1638	3285	1387	2694	3525
Standard Deviation	35.5	68.8	187.2	56.87	156	224.2
CV(%)	3.6	4.2	5.7	4.1	5.8	6.4

Resource

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H