Cxcl5 (Rat) ELISA Kit

Catalog Number KA1828

96 assays

Version: 01

Intended for research use only

I. Introduction

Rat LIX (LPS induced C-X-C chemokine) is a small secreted, non-glycosylated protein that belongs to the α-chemokine family. The molecule contains two intra-chain disulfide bonds and circulates as a non-disulfide linked 15 kDa homodimer. Rat LIX shares approximately 74% amino acid sequence identity with mouse LIX and is likely the rat orthologue to mouse LIX.

The Cxcl5 (Rat) ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of rat LIX in serum, plasma and cell culture supernatants. This assay employs an antibody specific for rat LIX coated on a 96-well plate. Standards and samples are pipetted into the wells and LIX present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-rat LIX antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of LIX bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. Reagents

1. LIX Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-rat LIX.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
3. Standards (Item C): 2 vials, recombinant rat LIX.
4. Assay Diluent A (Item D): 30 ml, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
5. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. For Standard/Sample (cell culture medium) diluent.
6. Detection Antibody LIX (Item F): 2 vial of biotinylated anti-rat LIX (each vial is enough to assay half microplate).
7. HRP-Streptavidin Concentrate (Item G): 8 µl of 20,000x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.
9. Stop Solution (Item I): 8 ml of 2 M sulfuric acid.

III. Storage

May be stored for up to 6 months at 2 to 8℃ from the date of shipment. Standard (recombinant protein) should be stored at -20℃ or -80℃ (recommended at –80℃) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8℃. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Note: the kit can be used within one year if the whole kit is stored at -20℃. Avoid repeated freeze-thaw cycles.

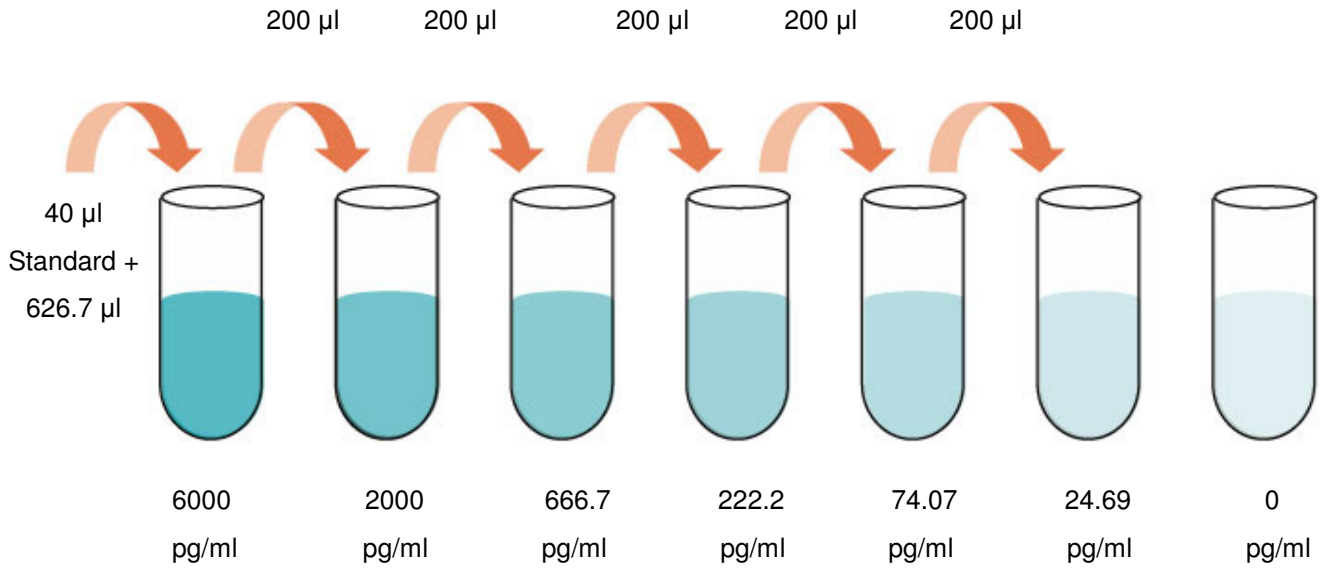
IV. Additional Materials Required

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.

3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

V. Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25℃) before use.
2. Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) is used for dilution of serum/plasma samples, and Assay. Diluent B (Item E) is used for dilution of culture supernatants.
3. Assay Diluent B should be diluted 5-fold with deionized or distilled water.
4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium) into Item C vial to prepare a 0.1 µg/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 40 µl LIX standard from the vial of Item C, into a tube with 626.7 µl Assay Diluent A or 1x Assay Diluent B to prepare a 6000 pg/ml stock standard solution. Pipette 400 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).



5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4℃ for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay

Diluent B and used in step 4 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 20,000-fold with 1x Assay Diluent.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 µl of HRP-Streptavidin concentrate into a tube with 198.0 µl 1x Assay Diluent B to prepare a 100-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix through and then pipette 70 µl of prepared 100-fold diluted solution into a tube with 14 ml 1x Assay Diluent B to prepare a final 20,000 fold diluted HRP-Streptavidin solution.Cell lysate buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

VI. Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25℃) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4℃ with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette 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.
4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.

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2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or over night at 4°C.

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3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.

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4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

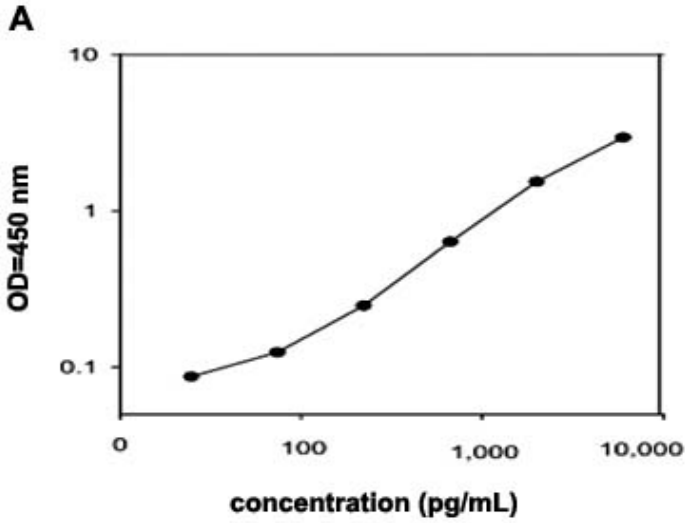
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

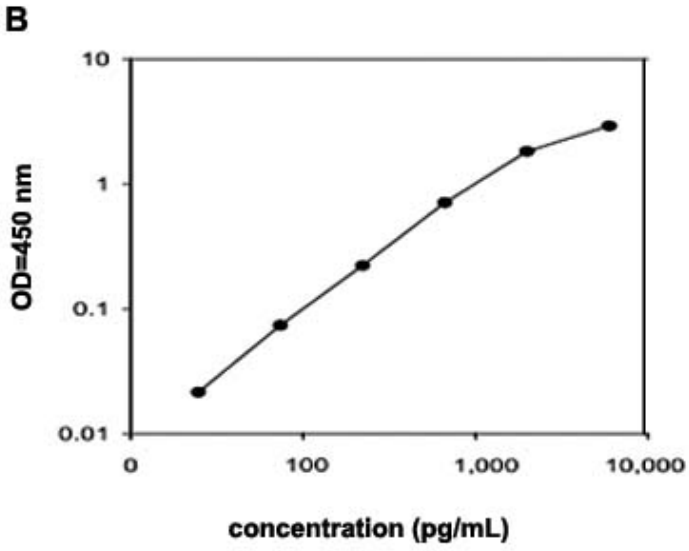
VIII. Calculation Of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.





B. Sensitivity

The minimum detectable dose of LIX is typically less than 20 pg/ml.

C. RECOVERY

Recovery was determined by spiking various levels of rat LIX into rat serum, plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	88.63	83-102
Plasma	89.45	84-103
Cell culture media	93.53	85-103

D. LINEARITY

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of	89	89	90
	Expected Range (%)	81-101	82-102	87-103
1:4	Average % of	92	94	93
	Expected Range (%)	84-103	83-104	84-105
1:8	Average % of	92	90	91
	Expected Range (%)	85-104	85-105	83-108

E. REPRODUCIBILITY

Intra-Assay: CV<10%

Inter-Assay: CV<12%

IX. SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., rat CINC-2, CINC-3, CNTF, Fractalkine, IL-1α, IL-1β, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, Leptin, MCP-1, MIP-3α, β-NGF, TIMP-1, TNF-α, VEGF)

X. REFERENCES

1. Rovai LE et al. (1997) Journal of Immunology 158 (11): 5257-66.
2. Zlotnik, A. and O. Yoshie (2000) Immunol. 78: 57.
3. Smith JB and Herschman HR (1995). Journal of Biological Chemistry 270(28): 16756-65.

XI. Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
Low signal	Too brief incubation times	Ensure sufficient incubation time; assay procdure step 2 change to over night
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at<-20℃ after reconstitution, others at 4℃. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measure