Mouse LIX/CXCL5 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE10070

Size: 96T

Sensitivity: 19.3 pg/mL

Range: 31.25-2000 pg/mL

Usage: For the quantitative detection of mouse LIX/CXCL5 concentrations in serum, plasma and tissue lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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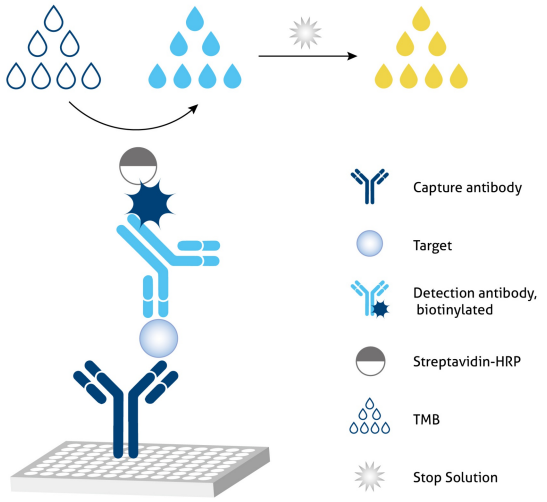
1. Background

LPS-induced CXC chemokine (LIX) is a murine chemokine similar to two human chemokines, ENA-78 (CXCL5) and GCP-2 (CXCL6). Mouse LIX can be produced by many immune cells, such as macrophages, eosinophils , as well as non-immune cells including mesothelial cells, and fibroblasts. Increased serum LIX has been observed in obesity and insulin resistance in mice and humans and is associated with complications of diabetes including nephropathy and atherosclerosis. It is mainly produced when induced by LPS, IL-17 and/or TNF-α. According to the previous studies, cancer cell autonomous secretion of LIX as sufficient to drive infiltration of mature, protumorigenic neutrophils in a mouse model of non-small cell lung cancer (NSCLC).

2. Principle



Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 2000 pg/bottle; lyophilized	2 bottles	
Detection Antibody (100×), biotinylated - 120 µL/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 3 - 30 mL/bottle. For mouse serum and plasma	1 bottle	
Sample Diluent PT 3-ec - 30 mL/bottle. For cell culture supernatant and tissue lysate	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

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6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

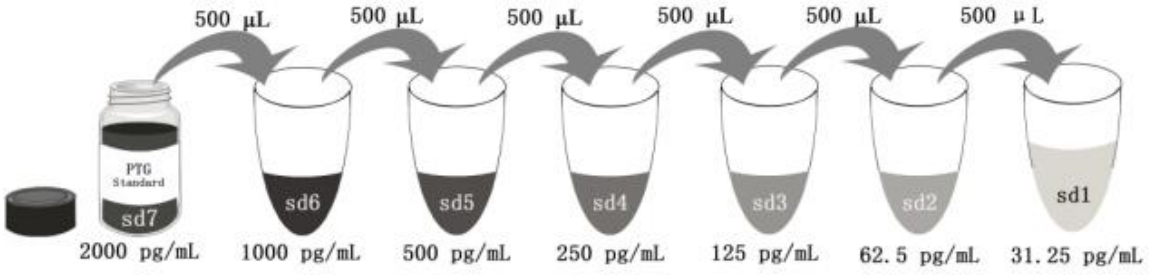
7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Streptavidin-HRP + 990 µL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:40 or 1:80 is recommended for mouse serum and plasma; 1:2 or 1:4 is recommended for cell culture supernatant.1:20 or 1:40 is recommended for tissue lysate.

7.5 Standard Serial Dilution:

For mouse serum and plasma samples, add 1 mL Sample Diluent PT 3 in protein standard; For cell culture supernatant and tissue lysate, add 1 mL Sample Diluent PT 3-ec in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3 or PT 3-ec	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). 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.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X Streptavidin-HRP solution (refer to Reagent Preparation7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C .

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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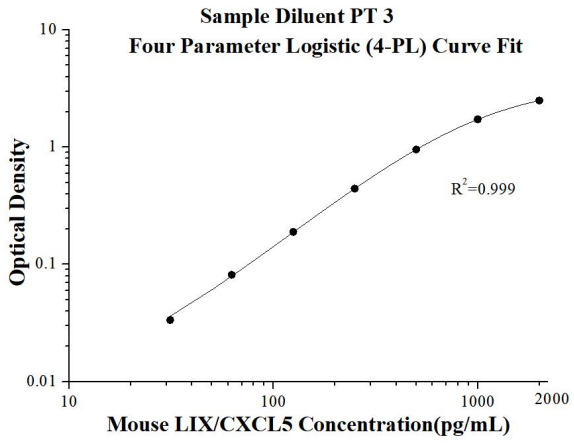
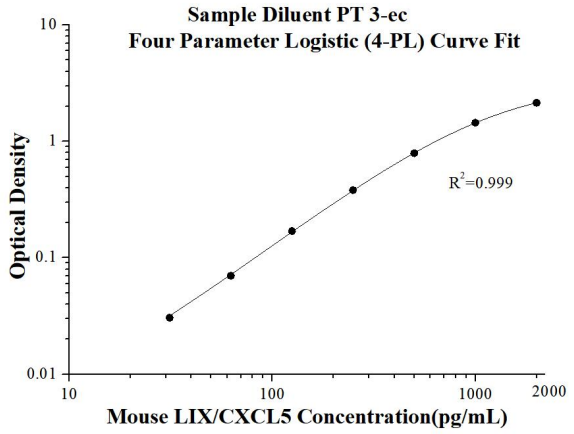
9. Validation Data

9.1 Standard curve

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



(pg/mL)	O.D	Average	Corrected
0	0.051 0.051	0.051	-
31.25	0.084 0.085	0.085	0.034
62.5	0.133 0.132	0.133	0.082
125	0.246 0.234	0.240	0.189
250	0.485 0.503	0.494	0.443
500	0.973 1.036	1.005	0.954
1000	1.729 1.826	1.778	1.727
2000	2.506 2.584	2.545	2.494



(pg/mL)	O.D	Average	Corrected
0	0.077 0.079	0.078	-
31.25	0.109 0.108	0.109	0.031
62.5	0.151 0.145	0.148	0.070
125	0.254 0.241	0.248	0.170
250	0.487 0.432	0.460	0.382
500	0.898 0.838	0.868	0.790
1000	1.491 1.553	1.522	1.444
2000	2.235 2.212	2.224	2.146

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	930.7	78.7	8.5
2	20	223.9	6.9	3.1
3	20	47.7	4.8	10.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	787.0	50.9	6.5
2	24	180.4	8.4	4.7
3	24	31.4	1.8	5.7

9.3 Recovery

The recovery of mouse LIX/CXCL5 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:150	115	108-124
	1:300	111	102-117
Tissue lysate	1:40	104	91-119
	1:80	102	85-114
Cell culture supernatant	1:4	106	82-113

9.4 Sample values

Mouse serum -The mouse serum samples were evaluated for the presence of mouse LIX/CXCL5 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Mouse serum (n=12)	23,245	14,361-38,673

Tissue lysate - Organs from 2-3 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized with a tissue homogenizer, and **Extraction Reagent** was added. An aliquot of each tissue lysate was removed and assayed for levels of mouse LIX/CXCL5.

Tissue Type	(pg/mL)
Lung	5,302
Spleen	6,156

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9.5 Sensitivity

The minimum detectable dose of mouse LIX/CXCL5 is 19.3 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, cell culture supernatant samples were spiked with high concentrations of mouse LIX and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Mouse serum and tissue lysate were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The mouse serum were initially diluted 1:10, the tissue lysates were initially diluted 1:10）
(The mouse serum was initially diluted 1:20. The tissue lysate was initially diluted 1:10）

		Mouse serum (Sample DiluentPT 3)	Tissue lysate (Sample Diluent PT 3-ec)	Cell culture supernatant (Sample Diluent PT 3-ec)
1:2	Average% of Expected	100	100	121
	Range (%)	-	-	120-121
1:4	Average% of Expected	106	109	104
	Range (%)	106-107	107-111	99-109
1:8	Average% of Expected	107	119	99
	Range (%)	106-108	116-121	93-105
1:16	Average% of Expected	108	117	90
	Range (%)	104-113	111-123	81-99

10. References

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