OriGene Technologies Inc.
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Mouse CXCL5 / LIX / ENA-78 / GCP 2 Fast ELISA Kit

Catalog Number: EA102932

Assay Principle

The OriGene Mouse Cxcl5 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Mouse Cxcl5 with a 96-well strip plate that is pre-coated with antibody specific for Cxcl5. The detection antibody is a biotinylated antibody specific for Cxcl5. The capture antibody is monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Mouse Cxcl5 with immunogen: Expression system for standard: E.coli; Immunogen sequence: V45-A118. The kit is analytically validated with ready to use reagents.

To measure Mouse Cxcl5, 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 Mouse Cxcl5 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 Mouse Cxcl5 in the sample.

Overview

Product Name	Mouse CXCL5 / LIX / ENA-78 / GCP 2 Fast ELISA Kit
Reactive Species	Mouse
Size	96wells/kit, with removable strips.
Description	The Fast version of ELISA kits, assay takes less than 1.5 hours. Detect Mouse Ena-78/CXCL5 with <10pg/ml sensitivity. Format: 96-well plate with removable strips. Compatible samples: cell culture supernates, serum and plasma(heparin, EDTA, citrate). This is a TMB colorimetric sandwich ELISA kit with short assay time and fast experiment set up. Ena-78/CXCL5 tissue specificity:
Sensitivity	<10pg/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.
Detection Range	15.6pg/ml-1000pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Uniprot ID	P50228

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Technical Details

Capture/Detection Antibodies	*The capture antibody is monoclonal antibody from rat, the detection antibody is polyclonal antibody from goat.*
Specificity	*Natural and recombinant Mouse Cxcl5*
Immunogen	*Expression system for standard: E.coli; Immunogen sequence: V45-A118*
Cross Reactivity	*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
Anti-tag Pre-coated 96-well strip microplate	**1**	**12 strips of 8 wells**
Mouse Cxcl5 Standard	**2**	**10ng/tube**
Mouse Cxcl5 Antibody Cocktail	**1**	**6ml**
Sample Diluent	**1**	**15ml**
TBS-T Wash Buffer (25x)	**1**	**12ml**
Color Developing Reagent (TMB)	**1**	**10ml**
Stop Solution	**1**	**10ml**
Plate Sealers	**2**	**Piece**

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Required Materials That Are Not Supplied

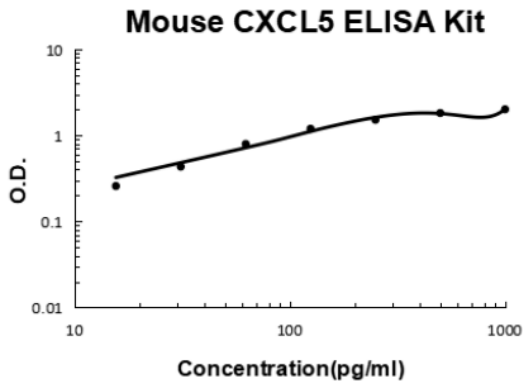
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml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

Mouse CXCL5 / LIX / ENA-78 / GCP 2 Fast ELISA Kit (EA102932) Standard Curve

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	15.6	31.2	62.5	125	250	500	1000
O.D.	0.005	0.263	0.439	0.800	1.200	1.524	1.826	2.017

Mouse CXCL5/ENA-78 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.*

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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)	35	166	388	36	150	360
Standard deviation	2.17	8.13	16.93	2.48	8.1	20.52
CV(%)	6.2%	4.9%	4.7%	6.9%	5.4%	5.7%

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	35	34	32	34	33	1.08	3.2%
Sample 2	166	180	162	152	165	10.04	6%
Sample 3	388	401	390	458	409	28.57	6.9%

**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. Also the TMB incubation time estimate (20-25min) is based on 37°C.
Wash buffer	Add 10ml of Wash Buffer into 240ml of deionized water.
Mouse Cxcl5 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 Mouse Cxcl5 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml 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.

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Dilution of Mouse Cxcl5 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –1000pg/ml, #2 –500pg/ml, #3 – 250pg/ml, #4 – 125pg/ml, #5 – 62.5pg/ml, #6 – 31.25pg/ml, #7 – 15.625pg/ml, #8 – 0.0 (Blank).
2. To generate standard #1, add 100µl of the reconstituted standard stock solution of 10ng/ml and 900µl 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, EDTA or citrate 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

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.

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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 50 µl of the standard, samples, or control per well. Add 50µl of Sample Diluent into the Zero well. At least two replicates of each standard, sample, or control is recommended.*
4. *And add 50µl of Mouse Cxcl5 Antibody Cocktail per well.*
5. *Cover with the plate sealer provided and incubate for 60 minutes at RT.*
6. *Wash the plate 4 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 90 seconds between each wash).*
   c. *Repeat steps a-b 2 additional times.*
   d. *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.*
7. *Add 90 µl of Color Developing Reagent to each well and incubate in the dark for 15 minutes at RT. (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.)*
8. *Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.*
9. *Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.*

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.

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.

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

Background on Cxcl5

C-X-C motif chemokine 5 is a protein that in humans encoded by the CXCL5 gene. The protein encoded by this gene, CXCL5 is a small cytokine belonging to the CXC chemokine family that is also known as epithelial-derived neutrophil-activating peptide 78 (ENA-78). It is produced following stimulation of cells with the inflammatory cytokines interleukin-1 or tumor necrosis factor-alpha. Expression of CXCL5 has also been observed in eosinophils, and can be inhibited with the type II interferon IFN-gamma. This chemokine stimulates the chemotaxis of neutrophils possessing angiogenic properties. It elicits these effects by interacting with the cell surface chemokine receptor CXCR2. The gene for CXCL5 is encoded on four exons and is located on human chromosome 4 amongst several other CXC chemokine genes. CXCL5 has been implicated in connective tissue remodeling. CXCL5 plays a role in reducing sensitivity to sunburn pain in some subjects, and is a potential target which can be utilized to understand more about pain in other inflammatory conditions like arthritis and cystitis.