Mouse Leptin ELISA Kit, pink-ONE

1. Catalog No. K0331250P

2. Quantity 96 tests

3. Storage 4°C

4. Description Mouse Leptin ELISA kit contains all the necessary reagents required for performing quantitative measurement of Mouse Leptin levels from samples including serum, plasma, culture medium or other biological fluids in a sandwich ELISA format.

5. Standard range 20-1250 pg/ml

6. Kit Contents

Component	Description	Amount
Pre-Coated 96 well ELISA microplate	Antigen-affinity purified Rabbit anti-Mouse Leptin pre-coated 96well plate	1 Plate
Detection Antibody (Lyophilized)	Biotinylated antigen-affinity purified Rabbit anti-Mouse Leptin	1 EA
Standard Protein (Lyophilized)	Recombinant Mouse Leptin	1 EA
Color Development Enzyme	Streptavidin-HRP conjugate (600 ul)	1 EA
pink-ONE Assay Diluent	0.1% Casein in PBS (50 ml)	1 EA
Prestained Color Development Reagent	pink-ONE TMB solution (10 ml)	1 EA
Stop Solution	2M H_2SO_4 (10 ml)	1 EA
PBS powder	Pouch for 1 L	1 EA
Tween-20 (50%)	1 ml	1 EA
Plate Sealer		3 EA

7. Reconstitution & Storage

1. Mouse Leptin Standard: 100 ng (1 vial) of recombinant Mouse Leptin should be reconstituted in 100 ul sterile water for a concentration of 1 ug/ml.

2. Detection Antibody: 10 ug (1 vial) of biotinylated antigen-affinity purified anti-Mouse Leptin should be reconstituted in 250 ul sterile water for a concentration of 40 ug/ml.

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Note: Reconstituted solutions are stable at -20°C for up to 2 months. Do not repeat frozen and thawing.

8. Reagent Preparations

*** All preparations should be mixed thoroughly and warmed up at room temperature prior to use.**

1. Washing Solution (PBST): Resolve the PBS powder (1 pouch) to sterile water and make 1 Liter, then add 1 ml Tween-20 (50%) to this solution and mix well.

2. Pre-coated ELISA 96 well plate: Select the number of coated wells required for the assay. The remaining wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

3. Standards
   Dilute the standards and samples in pink-ONE Assay Diluent at 1:2 serial dilutions as follows:

Step	Dilution Method	Standard conc.
Step A	1.25 ul of Standard + 1 ml of Assay Diluent	1250 pg/ml
Step B	0.5 ml of Step A + 0.5 ml of Assay Diluent	625 pg/ml
Step C	0.5 ml of Step B + 0.5 ml of Assay Diluent	312.5 pg/ml
Step D	0.5 ml of Step C + 0.5 ml of Assay Diluent	156.25 pg/ml
Step E	0.5 ml of Step D + 0.5 ml of Assay Diluent	78.125 pg/ml
Step F	0.5 ml of Step E + 0.5 ml of Assay Diluent	39.0625 pg/ml
Step G	0.5 ml of Step F + 0.5 ml of Assay Diluent	19.53 pg/ml

4. Sample dilution: Dilute the samples to a proper concentration in pink-ONE Assay Diluent.

   Note: Dilute the samples, based on the expected concentration of the analyte, to fall within the concentration range of the standards.

5. Detection Antibody: Dilute the reconstituted detection antibody in pink-ONE Assay Diluent to a concentration of 1 ug/ml (1:40 dilution).

6. Color Development Enzyme: Dilute the Streptavidin-HRP conjugate 1:20 in pink-ONE Assay Diluent.

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9. Cautions

1. Store all solutions at 4°C and keep them from contamination.
2. All samples and kit reagents should be at room temperature (20-25°C) prior to use.
3. Vigorous washing of the plate after incubation steps is essential to obtaining low background values.
4. Dissolve antigen, standard and antibody perfectly.
5. Use clean pipet tips for each transfer to avoid cross contamination.
6. Stop solution (H_2SO_4) is a caustic material. Eye, hand, face, and clothing protection should be worn when handling this material.
7. Individual components of this kit contain no preservatives

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10. ELISA Protocol

1. Add 200 ul of Washing Solution to each well. Aspirate the wells to remove liquid and wash the plate 3 times using 300 ul of Washing Solution per well. After the last wash, invert plate to remove residual solution and blot on paper towel.

   Note: Do not dry the well completely and so immediately go on next step.

2. Add 100 ul of standard or sample to each well in duplicate. Cover with the Plate Sealer provided. Incubate at room temperature for at least 2 hours.

3. Aspirate the wells to remove liquid and wash the plate 4 times like as step 1.

4. Add 100 ul of the diluted detection antibody (1 ug/ml) per well. Cover with the Plate Sealer provided. Incubate at room temperature for 2 hours.

5. Aspirate and wash plate 4 times like as step 1.

6. Add 100 ul of the diluted Color Development Enzyme (1:20 dilute) per well. Cover with the Plate Sealer provided. Incubate 30 minutes at room temperature (or 37°C for 30 minutes).

7. Aspirate and wash plate 4 times like as step 1.

8. Add 100 ul of pink-ONE TMB Color Development Reagent to each well. Incubate at room temperature for a proper color development (10-20 minutes). pink-ONE TMB produces a deep blue color during the enzymatic degradation of H_2O_2 by peroxidase. To stop the color reaction, add 100 ul of the stop solution to each well.

   Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

   Note: According to the reaction intensity, the color changes to violet, then deep blue during a reaction.

9. Using a microtiter plate reader, read the plate at 450 nm wavelength.

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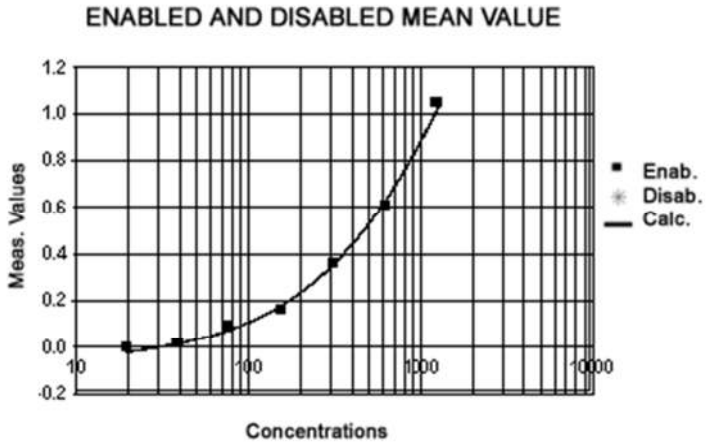
11. Calculation of Results

1. Average the duplicate readings from each standard, control, and samples.

2. Subtract the zero reading from each averaged value above.

3. Create a standard curve by reducing the data using ELISA reader's computer software capable of generating Standard curve-fit.
   * A standard curve should be generated for each set of samples (See example).



Mouse Leptin (pg/ml)
(15 minutes color development)

12. Cross Reactivity

When tested at 50 ng/ml the following antigen(recombinant protein) did not exhibit significant cross reactivity:

Host	Tested Antigen (recombinant protein)
Human	GLP-1, IL-1α, IL-1β, Resistin, Visfatin
Mouse	IL-1α, IL-1β, Resistin
Rat	IL-1α, IL-1β

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13. Trouble shooting

Problem	Probable Cause	Solution
Low O.D.	Reagents not fresh or contamination	Ensure reagents have been prepared correctly and are best before date.
	Incubation time not long enough	Ensure you are incubating the antibody for the recommended amount of time, if an incubation time is suggested.
	Incubation temperature too low	incubators are set in the correct temperature and working. Ensure all reagents are at room temperature before proceeding.
	Stop solution not added	Addition of stop solution
High O.D.	Standard reconstituted with less volume than required	Reconstitute lyophilized standard with correct volume of solution recommended in the protocol.
	Detection antibody, Streptavidin-HRP, Substrate solutions incubation times are too long	Decrease incubation time.
Poor Duplicates	Plate washing was not adequate or uniform	Make sure pipette tips are tightly adjusted. Confirm all reagents are removed completely in all wash steps.
	Not mixed well sample	Thoroughly mix samples before pipetting.
	Samples may have high particulate density matter	Remove the particulate matter by centrifugation.
	Cross-well contamination	Do not use used plate sealers Do not use used pipette tips
High background	Contamination of reagents/samples	Use fresh reagents/samples and pipette carefully.
	Insufficient plates washing	Ensure well areas are washed adequately by filling the wells with wash buffer.
	Too much antibody used leading to non-specific binding	Try to use less antibody.
	Streptavidin-HRP too strong or left on too long exposure	Check dilution of conjugate, use it at the recommended dilution.
	Substrate solution or stop solution is not fresh	Use fresh substrate solution.
	Plate left too long before reading on the plate reader	Color will keep developing (though at a slower rate if stop solution has been added).
	Incubation temperature is too high	incubators are set in the correct temperature and working.
Sample readings are out of range	Samples contain no or below detectable levels of analyte or Samples contain analyte concentrations greater than the highest standard point.	If samples are below detectable levels, it may be possible to use higher sample volume. If samples are high detectable levels, it may require dilution and reanalysis.

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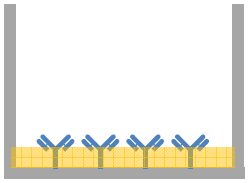
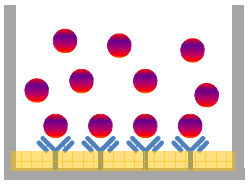
Summary of the ELISA procedure

: Capture Antibody
: Antigen
: Detection Antibody - Biotin
: Streptavidin-HRP



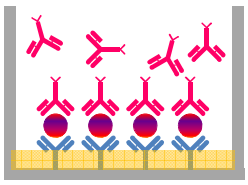
Precoated Plate

Wash the plate 4 times with PBST



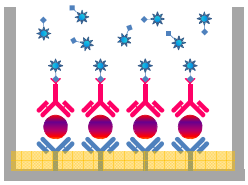
Add Standard or Samples and incubate for 2 hr at room temperature.

Wash the plate 4 times with PBST



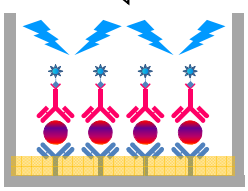
Add Detection Antibody and incubate for 2 hr at room temperature.

Wash the plate 4 times with PBST



Add Streptavidin-HRP and incubate for 30 min at room temperature.

Wash the plate 4 times with PBST



Color development with TMB substrate and read the plate at 450 nm wavelength

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Related Products : KOMA Cytokine ELISA Kit, pink-ONE

Cat. No.	Description
K0331251P	BD-1 Human
K0331208P	BD-2 Human
K0331254P	CNTF Human
K0331188P	Eotaxin-3 Human
K0331115P	EGF Human
K0331228P	EGF Mouse
K0331192P	bFGF Human
K0331226P	G-CSF Mouse
K0331120P	GM-CSF Human
K0331137P	GM-CSF Mouse
K0332101P	HGF Human
K0332112P	IGF-I Human
K0331225P	IGF-I Mouse
K0331121P	IFN-gamma Human
K0331138P	IFN-gamma Mouse
K0331209P	IFN-gamma Rat
K0331210P	IP-10 Human
K0331255P	IP-10 Mouse
K0331125P	IL-1 alpha Human
K0331141P	IL-1 alpha Mouse
K0331211P	IL-1 alpha Rat
K0331800P	IL-1 beta Human
K0331231P	IL-1 beta Mouse
K0331212P	IL-1 beta Rat
K0331193P	IL-2 Human
K0331142P	IL-2 Mouse
K0332100P	IL-2 Rat
K0331126P	IL-3 Human
K0331143P	IL-3 Mouse
K0331214P	IL-4 Human
K0331144P	IL-4 Mouse
K0331127P	IL-5 Human
K0331194P	IL-6 Human
K0331230P	IL-6 Mouse
K0331229P	IL-6 Rat
K0331215P	IL-7 Human
K0331216P	IL-8 Human
K0331232P	IL-9 Human
K0331123P	IL-10 Human
K0331213P	IL-10 Mouse
K0331124P	IL-12 Human
K0331139P	IL-12 Mouse

Cat. No.	Description
K0331235P	IL-13 Human
K0331201P	IL-13 Mouse
K0331260P	IL-15 Human
K0331207P	IL-17A Human
K0331198P	IL-17E Human
K0331190P	IL-20 Human
K0331236P	IL-21 Human
K0331234P	IL-22 Human
K0331233P	IL-31 Human
K0331253P	IL-33 Human
K0332133P	Rat IL-4
K0331250P	Leptin Mouse
K0331227P	M-CSF Mouse
K0331195P	MIP-1 alpha Human
K0331202P	MIP-1 alpha Mouse
K0331247P	MIP-1 alpha Rat
K0331252P	MIP-1 beta Mouse
K0331217P	MIP-2 Mouse
K0331218P	MCP-1 Human
K0331219P	MCP-1 Mouse
K0331220P	NGF-beta Human
K0331191P	PDGF-BB Human
K0331221P	RANTES Human
K0331222P	RANTES Mouse
K0331223P	RANTES Rat
K0331199P	Resistin Human
K0331187P	sRANK Ligand Human
K0331203P	sRANK Ligand Mouse
K0331130P	SCF Human
K0331148P	SCF Mouse
K0331204P	SCF Rat
K0331200P	TRAIL Human
K0332110P	TGF-beta 1 Human
K0332120P	TGF-beta 2 Human
K0332130P	TGF-beta 3 Human
K0331131P	TNF-alpha Human
K0331186P	TNF-alpha Mouse
K0331196P	TNF-alpha Rat
K0331132P	VEGF Human
K0331224P	VEGF Mouse
K0331268P	Mouse IL-17
K0332113P	Human Leptin

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