Human IL-27 ELISA Kit

1. Catalog No. K0332156

2. Quantity 96 tests

3. Storage 4°C

4. Description Human IL-27 ELISA kit contains all the necessary reagents required for performing quantitative measurement of Human IL-27 levels from samples including serum, plasma, culture medium or other biological fluids in a sandwich ELISA format.

5. Standard range 156-10000 pg/ml

6. Kit Contents

Component	Description	Amount
Pre-Coated 96 well ELISA microplate	Antigen-affinity purified Goat anti-Human IL-27 pre-coated 96well plate	1 Plate
Detection Antibody (Lyophilized)	Biotinylated antigen-affinity purified Goat anti-Human IL-27	2 EA
Standard Protein (Lyophilized)	Recombinant Human IL-27	2 EA
Color Development Enzyme	Streptavidin-HRP conjugate (600 ul)	1 EA
Assay Diluent	1% BSA in PBS (50 ml)	1 EA
Color Development Reagent	TMB solution (10 ml)	1 EA
Stop Solution	2M H_2SO_4 (10 ml)	1 EA
Wash Buffer (20X)	Concentrated PBST for 1L (50ml)	1 EA
Plate Sealer		3 EA

7. Reconstitution & Storage

1. Human IL-27 Standard: 11 ng (1 vial) of recombinant Human IL-27 should be reconstituted in 110 ul sterile water for a concentration of 0.1 ug/ml.

2. Detection Antibody: 0.55 ug (1 vial) of biotinylated antigen-affinity purified anti- Human IL-27 should be reconstituted in 0.275 ul sterile water for a concentration of 2 ug/ml.
   Note: Reconstituted solutions are stable at -20°C for up to 2 months. Avoid repeated freezing and thawing after reconstitution.

For research use only; not for use as a diagnostic

KOMABIOTECH
koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

8. Reagent Preparations

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

1. Washing Solution (PBST): Dilute Wash Buffer at 1:20 in sterile water 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 Assay Diluent at 1:2 serial dilutions as follows:

Step	Dilution Method	Standard conc.
Step A	10 ul of Standard + 0.99 ml of Assay Diluent	10000 pg/ml
Step B	0.5 ml of Step A + 0.5 ml of Assay Diluent	5000 pg/ml
Step C	0.5 ml of Step B + 0.5 ml of Assay Diluent	2500 pg/ml
Step D	0.5 ml of Step C + 0.5 ml of Assay Diluent	1250 pg/ml
Step E	0.5 ml of Step D + 0.5 ml of Assay Diluent	625 pg/ml
Step F	0.5 ml of Step E + 0.5 ml of Assay Diluent	312.5 pg/ml
Step G	0.5 ml of Step F + 0.5 ml of Assay Diluent	156.25 pg/ml

   Note: Transfer 100 ul of Assay Diluent to empty well for Standard Blank.

4. Sample dilution: Dilute the samples to a proper concentration in 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 Assay Diluent to a concentration of 0.1 ug/ml (1:20 dilution).

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

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

biotechnology
KOMABIOTECH

koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

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 (0.1 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 color development solution to each well. Incubate at room temperature for a proper color development. (5-15 minutes) 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.

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

biotechnology
KOMABIOTECH

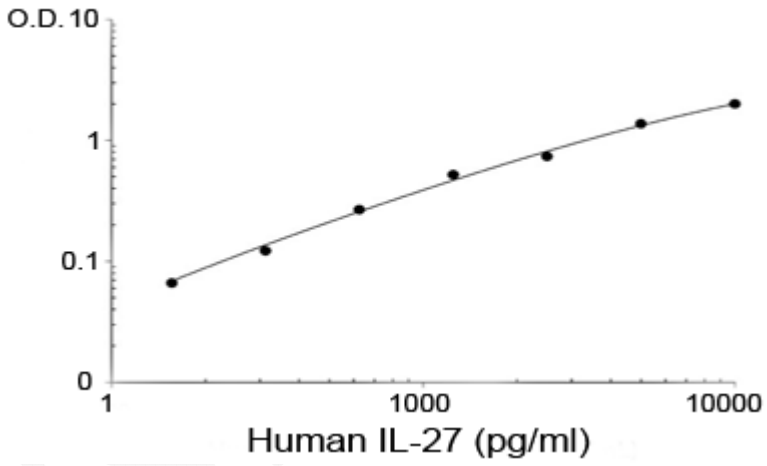
koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

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).



Human IL-27 (pg/ml)
(10 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	IL-12 p70, IL-12/IL23 p40
Mouse	IL-27

KOMABIOTECH

koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

13. Trouble shooting

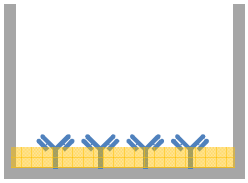
Problem	Probable Cause	Solution
Low O.D.	Reagents not fresh or contamination	Ensure reagents have been prepared correctly and are best before date.
	Incubation time not long enough	Ensure you are incubating the antibody for the recommended amount of time, if an incubation time is suggested.
	Incubation 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.

biotechnology
KOMABIOTECH

koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

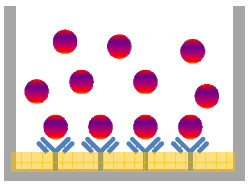
Summary of the ELISA procedure

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



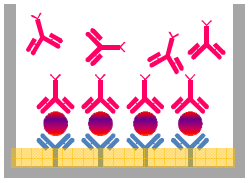
Precoated Plate

Wash the plate 4 times with PBST



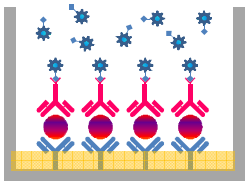
Add Standard or Samples and incubate for 2 hr at room temperature.

Wash the plate 4 times with PBST



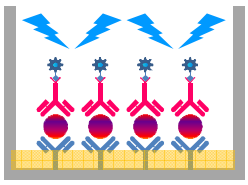
Add Detection Antibody and incubate for 2 hr at room temperature.

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



biotechnology
KOMABIOTECH
koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

Related Products : KOMA Cytokine ELISA Kit

Cat. No.	Description
K0331251	BD-1 Human
K0331208	BD-2 Human
K0331254	CNTF Human
K0331188	Eotaxin-3 Human
K0331115	EGF Human
K0331228	EGF Mouse
K0331192	bFGF Human
K0331226	G-CSF Mouse
K0331120	GM-CSF Human
K0331137	GM-CSF Mouse
K0332101	HGF Human
K0331225	IGF-I Mouse
K0331121	IFN-gamma Human
K0331138	IFN-gamma Mouse
K0331209	IFN-gamma Rat
K0331210	IP-10 Human
K0331125	IL-1 alpha Human
K0331141	IL-1 alpha Mouse
K0331211	IL-1 alpha Rat
K0331800	IL-1 beta Human
K0331231	IL-1 beta Mouse
K0331212	IL-1 beta Rat
K0331193	IL-2 Human
K0331142	IL-2 Mouse
K0332100	IL-2 Rat
K0331126	IL-3 Human
K0331143	IL-3 Mouse
K0331214	IL-4 Human
K0331144	IL-4 Mouse
K0331127	IL-5 Human
K0331194	IL-6 Human
K0331230	IL-6 Mouse
K0331229	IL-6 Rat
K0331216	IL-8 Human
K0331232	IL-9 Human
K0331123	IL-10 Human
K0331213	IL-10 Mouse
K0331124	IL-12 Human
K0331139	IL-12 Mouse
K0331235	IL-13 Human
K0331201	IL-13 Mouse
K0331260	IL-15 Human
K0331207	IL-17A Human
K0331198	IL-17E Human
K0331190	IL-20 Human
K0331236	IL-21 Human
K0331234	IL-22 Human
K0331233	IL-31 Human
K0331253	IL-33 Human

Cat. No.	Description
K0331252	MIP-1 beta Mouse
K0331217	MIP-2 Mouse
K0331218	MCP-1 Human
K0331219	MCP-1 Mouse
K0331220	NGF-beta Human
K0331191	PDGF-BB Human
K0331221	RANTES Human
K0331222	RANTES Mouse
K0331223	RANTES Rat
K0331199	Resistin Human
K0331187	sRANK Ligand Human
K0331203	sRANK Ligand Mouse
K0331130	SCF Human
K0331148	SCF Mouse
K0331204	SCF Rat
K0331200	TRAIL Human
K0332110	TGF-beta 1 Human
K0332120	TGF-beta 2 Human
K0332130	TGF-beta 3 Human
K0331131	TNF-alpha Human
K0331186	TNF-alpha Mouse
K0331196	TNF-alpha Rat
K0331132	VEGF Human
K0331224	VEGF Mouse
K0331268	IL-17 Mouse
K0332133	IL-4 Rat
K0331263	EGF Rat
K0332140	NGF-beta Rat
K0331269	IL-21 Mouse
K0331265	G-CSF Human
K0332151	Human IL-23
K0331281	ICAM-1 Human
K0331271	BD-3 Human
K0331267	MCP-1 Rat
K0331261	VEGF Rat
K0332146	Mouse IL-15
K0332134	IL-10 Rat
K0331270	MCP-3 Mouse
K0332131	TNF-beta Human
K0332165	TRAIL Mouse
K0332150	Human sTNF RII
K0332132	Rat GM-CSF
K0331215	Human IL-7
K0332163	Mouse IL-9
K0332142	Mouse IL-23
K0332136	Mouse IL-5
K0332152	Human BDNF
K0332138	Human M-CSF
K0332158	Human TGF-alpha

For research use only; not for use as a diagnostic

biotechnology
KOMABIOTECH

koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669

Color development with TMB substrate and read the plate at 450

K0332113	Leptin Human
K0331250	Leptin Mouse
K0331227	M-CSF Mouse
K0331195	MIP-1 alpha Human
K0331202	MIP-1 alpha Mouse
K0331247	MIP-1 alpha Rat

K0331273	Human Eotaxin
K0331274	Human BMP-2
K0332144	IL-22 Mouse
K0331255	IP-10 Mouse
K0332112	IGF-I Human
K0332156	IL-27 Human

KOMABIOTECH

koma@komabiotech.com
Tel. 82-2-2660-5660 Fax. 82-2-2660-5669