Human LL-37 ELISA kit

Cat#: BG310083

Size: 96T

Assay Principle

The Human LL-37 ELISA Kit is designed to detect and quantify the level of Human LL-37 in serum, plasma, and other biological fluids.

This ELISA kit uses the Sandwich-ELISA principle. The microplate provided in this kit has been pre-coated with an antibody specific to Human LL-37. Samples (or Standards) are added to the microplate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human LL-37 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human LL-37, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450±2 nm. The OD value is proportional to the concentration of Human LL-37. You can calculate the concentration of Human LL-37 in the samples by comparing the OD of the samples to the standard curve.

Contents and Storage

An unopened kit can be stored at 2-8℃ for 12 months. After opening, store the items separately according to the conditions specified in the table below.

Components	Quantity (96 tests)	Storage
LL-37 Antibody Coated Microplate	8 wells x 12 strips	-20℃, 12 months
LL-37 Standard	2 vials	-20℃, 12 months
LL-37 Biotinylated Detection Antibody (100X)	120 µL	-20℃, 12 months
HRP Conjugate (100X)	120 µL	-20℃ (Protect from light), 12 months
Standard & Sample Diluent	20 mL	2-8℃, 12 months
Biotinylated Detection Antibody Diluent	14 mL	2-8℃, 12 months
HRP Conjugate Diluent	14 mL	2-8℃, 12 months
Wash Buffer Concentrate (25X)	30 mL	2-8℃, 12 months
Substrate Reagent	10 mL	2-8℃ (Protect from light), 12 months
Stop Solution; contains 1 M H_2SO_4, **CAUSTIC**	10 mL	2-8℃, 12 months
Plate Sealer	5	

Required materials

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions
- Incubator capable of maintaining 37℃.

Sample Preparation

Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃ before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Plasma: Collect plasma using EDTA or heparin (EDTA-Na_2 is most recommended) as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.
Other biological fluids: Centrifuge samples for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.
Note:

- Collect samples in pyrogen/endotoxin-free tubes.
- Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be aliquoted and stored at -20℃ (≤ 1 month) or -80℃ (≤ 3 months). Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge, or filter sample prior to analysis.

Note: Sample concentrations should be within the range of the standard curve. Because conditions may vary, the optimal dilution for each application should be determined. Use all prepared samples within 2 hours of dilution. It is not recommended to conduct experiments after 2 hours.

Reagent Preparation

Prepare 1X Wash Buffer

1. Dilute 30 mL of Wash Solution Concentrate (25X) with 720 mL of deionized or distilled water. Label as 1X Wash Buffer.

2. Store the concentrate and 1X Wash Buffer at 2-8°C. Use the diluted buffer within 3 months.
Note: if crystals have formed in the concentrate, warm it in a 40°C-water bath and mix it gently until the crystals have completely dissolved and the performance is not affected.

Prepare 1X Biotinylated Detection Antibody Solution

Note: The working solution should be prepared just before use

1. Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared.
2. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g at 2-8℃ for 1 min.
3. Dilute the Concentrated Biotinylated Detection Ab (100X) to 1X working solution with Biotinylated Detection Ab Diluent.

Prepare 1X HRP Conjugate Solution

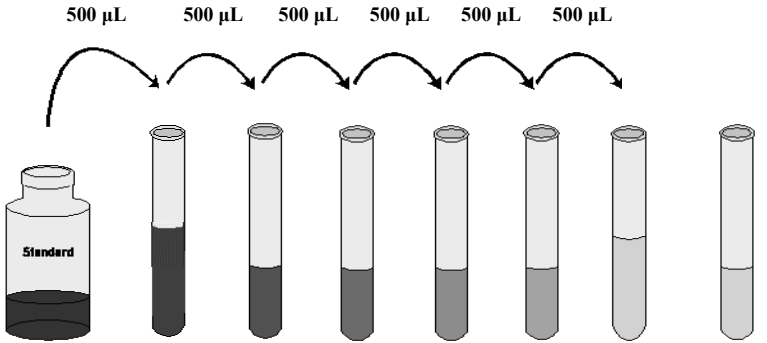
Note: The working solution should be prepared just before use

1. Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared.
2. Centrifuge the Concentrated HRP Conjugate at 800×g at 2-8℃ for 1 min.
3. Dilute the Concentrated HRP Conjugate (100X) to 1X working solution with HRP Conjugate Diluent.

Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

1. Centrifuge the standard at 10,000×g at 2-8℃ for 1 min to ensure the contents are at the bottom of vial.
2. Add 1 mL of Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. Once fully dissolved, mix it thoroughly with a pipette. This reconstitution produces a working solution of 100 ng/mL.
3. Take 7 tubes, add 500 μL of Standard & Sample Diluent to each tube. Pipette 500 μL of the 100 ng/mL working solution to the first tube and mix up to produce a 50 ng/mL working solution. Pipette 500 μL of the solution from the former tube into the latter one according to this step. The last tube is regarded as a blank, don't pipette solution into it from the former tube. The recommended dilution gradient is as follows: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/mL.
4. The illustration of diluted standards below is for reference. Mix thoroughly between dilution gradients.



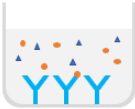
Diluent Volume	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
Std1	**Std2**	**Std3**	**Std4**	**Std5**	**Std6**	**Std7**	**Std8**
100 ng/mL	**50 ng/mL**	**25 ng/mL**	**12.5 ng/mL**	**6.25 ng/mL**	**3.13 ng/mL**	**1.56 ng/mL**	**0 ng/mL**

5. Use the diluted standards within 2 hours of preparation. If multiple standard tests are to be carried out, the redissolved standard solution (standard with the highest concentration) can be divided into 2-3 vials and frozen at -20℃, to be used within half a month. Avoid multiple freeze-thaw cycles.

Assay Procedure (Total assay time: 3.5 hours)

Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at -20℃ for future use. The silica pack in the bag keeps the plate dry.



1. Bind antigen

Note: solutions should be added to the bottom of the ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

a. For the standard curve, add 100 µL of standards to the appropriate wells. For samples, add 100 µL of pretreated samples to the wells.
b. Cover the plate with plate sealer and incubate for 90 min at 37℃.
c. Thoroughly aspirate the solution. Do not wash.

2. Add biotinylated detection antibody

a. Add 100 µL of **Biotinylated Detection Antibody Working Solution** into each well.
b. Cover the plate with plate sealer and incubate for 60 min at 37℃.
c. Thoroughly aspirate the solution and wash wells 3 times with 350 µL of 1X Wash Buffer. Decant the solution from each well, add 350 µL of **wash buffer** to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Proceed immediately to the next step, making sure the wells do not dry out.

3. Add HRP conjugate

a. Add 100 µL **HRP Conjugate Working Solution** into each well.
b. Cover the plate with plate sealer and incubate for 30 min at 37℃.
c. Thoroughly aspirate the solution and repeat the wash process for 5 times as conducted in step 2.

4. Add substrate

a. Add 90 µL **Substrate Reagent** to each well.
b. Cover the plate with plate sealer and incubate for about 15 min at 37℃. Protect the plate from light.

Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min.

5. Add stop solution

a. Add 50 µL **Stop Solution** to each well. This step should be done in the same order as the substrate solution. Tap the side of the plate gently to mix.
b. The solution in the wells will change from blue to yellow.

Data Analysis

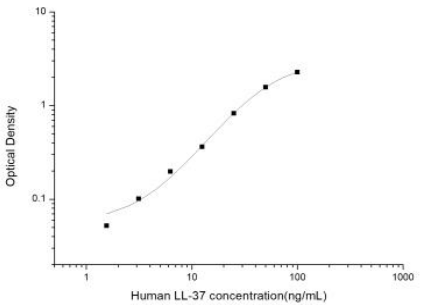
1. Preheat the Microplate Reader for about 15 min before OD measurement.
2. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
3. Use curve-fitting software to generate the standard curve. A four-parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns, and controls, prior to plotting.
4. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution.

Typical Data

▪ Standard curve (example)

Typical standard curve and data over the range of 0–100 ng/mL LL-37 is provided below for reference only.



- Expected values

Sixteen random Human serum/plasma samples were tested in the assay.

Sample Type	**LL-37** Range (**ng/mL**)	**LL-37** Average (**ng/mL**)
Serum (n=16)	2.52-4.265	3.31
Plasma (n=16)	8.23-38.25	16.8

Disclaimer

The reagent is only used in the field of scientific research , not suitable for clinical diagnosis or other purposes.