Human Zonulin ELISA Kit

to determine Human Zonulin in Serum, Blood Plasma, Saliva, Urine, Tissue Liquid Samples or related Biological Solutions.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTuH4073EA

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Toll Free: 1800-120-2434
Mobile: +91-98105-21400 | Fax: +91-11-42208444
customerservice@lifetechindia.com

INTENDED USE

This kit is used to determine Human Zonulin in the sample of serum, blood plasma, saliva, urine, tissue liquid samples or related biological solutions. For *in vitro* use only.

MANUAL VERSION 1.01

ASSAY SUMMARY

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human Zonulin antibody. Zonulin present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human Zonulin Antibody is added and binds to Zonulin in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated Zonulin antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human Zonulin. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

CHARACTERISTICS

- This Kit allows for the determination of ZONULIN concentrations in Human serum, cell culture supernates and other biological fluids.
- Detection range: 0.3-90 ng/ml.
- The minimum detectable dose (MDD) of Human ZONULIN is less than 0.13ng/ml.
- Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) < 8%.
- Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%) < 10%.
- To assess linearity of the assay, samples containing and/or spiked with high concentrations of Human ZONULIN were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

- Human ZONULIN ELISA Kit has high sensitivity and excellent specificity for detection of Human ZONULIN. No significant cross-reactivity or interference between Human ZONULIN and analogues was observed.

PRODUCT INFORMATION

MATERIALS SUPPLIED AND STORAGE CONDITIONS

Store kit reagents at 2-8 °C. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

Components	Quantity (96T)
Standard Solution (96ng/ml)	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human Zonulin Antibody	1ml x1
User Instruction	1
Plate Sealer	2 pics
Zipper bag	1 pic

Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

ASSAY PROTOCOL

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Note: Samples should be centrifuged adequately and no hemolysis or granule was allowed.

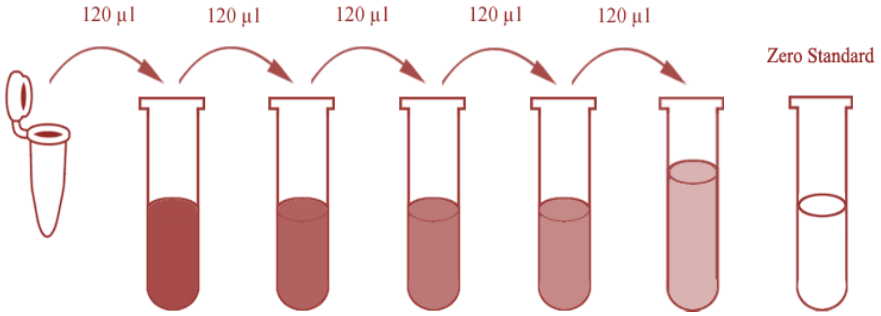
REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals were formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - Dilute with Distilled or deionized water 1:25.

- **Standard** - Reconstitute the 120µl of the standard (96ng/ml) with 120µl of standard diluent to generate a 48ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (48ng/ml) 1:2 with standard diluent to produce24ng/ml, 12ng/ml, 6ng/ml and3ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

48ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
24ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
12ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
6ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
3ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



ASSAY PROCEDURE

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells and then add 10µl anti-zonulin antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not to blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 mins after adding the stop solution.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Human ZONULIN concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

PRECAUTIONS

1. Do not mix or substitute reagents with those from other lots or sources.
2. This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
3. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

4. When mixing or reconstituting protein solutions, always avoid foaming.
5. 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.
6. When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
7. Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).