Human ITG beta2 / CD11+CD18 GENLISA™ ELISA

REF : KBH0237

Ver 1.0

RUO

Enzyme Immunoassay for the quantitative determination of Human ITG beta2 / CD11+CD18 in serum, plasma and cell culture supernatant

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

The Genlisa™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum / plasma as validated with the kit. The kit employs a sandwhich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

The Human ITG beta2 / CD11+CD18 GENLISA™ ELISA kit is used as an analytical tool for quantitative determination of Human ITG beta2 / CD11+CD18 in serum, plasma and cell culture supernatant.

Principle:

The method employs sandwhich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Human ITG beta2 / CD11+CD18 present in the sample are bound by the antibodies. Biotin labeled antibody is added and followed by HRP (horseradish peroxidase) conjugated secondary antibody is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human ITG beta2 / CD11+CD18 in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Coated Microtiter Plate (12x8 wells) – 1 no
2. Concentrated Standard 1600 pg/ml – 0.5 ml
3. Biotin Conjugate – 1ml
4. HRP Conjugate – 6 ml
5. Standard Diluent – 3ml
6. Wash Buffer (25X) – 20 ml
7. Substrate A – 6 ml
8. Substrate B – 6 ml
9. Stop Solution – 6 ml

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25µl to 1000µl
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. All reagents should be stored at 2ºC to 8ºC for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Cell Culture supernatant-** Detects secretory components. Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appeared, centrifuge again.
6. **Tissue samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

Note: *Grossly hemolyzed samples are not suitable for use in this assay.*

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X); dilute 20 ml of 25X Wash Buffer in 480 ml of DI water.
4. Standards Dilution: Dilute 120µl of original standard (1600 pg/ml) with 120µl of standard diluent to generate a 800 pg/ml standard stock solution. Keep the standard for 15 mins with gentle agitation before making dilutions. Prepare bynserially diluting the standard stock solution the standards as per the below table.

800 pg/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
400 pg/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
200 pg/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
100 pg/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
50 pg/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human ITG beta2 / CD11+CD18. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human ITG beta2 / CD11+CD18 present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the
3. Human ITG beta2 / CD11+CD18 concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.

4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human ITG beta2 / CD11+CD18.
5. It is recommended that all Standards and Samples be assayed in duplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **50µl standard** to standard well. Note: Don't add biotin conjugate to standard well because the standard solution contains biotinylated antibody.

3. Add **40µl sample** to sample wells and then add **10µl biotin conjgate** to sample wells, and then add **50µl HRP conjugate** to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate for **60 minutes** at **37°C.**

4. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 300µl wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.

5. Add **50µl substrate solution A** to each well and then add **50µl substrate solution B** to each well. Incubate plate covered with sealer for 10 minutes at 37°C in the dark.

6. Add **50µl Stop Solution** to each well, the blue color will change into yellow immediately.

7. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10-15 minuets after adding the stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Human ITG beta2 / CD11+CD18 Concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Human ITG beta2 / CD11+CD18 Concentration. If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit is best recommended for automated results.

Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

This kit has been validated. Please view the details herein below.

Standard Calibrator Concentration Provided | Standard Calibrator Graph Range | Sensitivity

Standard Calibrator = 1600 pg/ml; Standard Calibrator Range= 50 pg/ml – 800 pg/ml;
Sensitivity = 3.13 pg/ml.

Safety Precautions:

- **This kit is for research use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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