# Human βIG-H3 ELISA Kit

**(Catalog # K926-100; 100 assays; Store at -20°C)**

**I. Introduction:** BioVision's Human βIG-H3 (also known as Kerato-epithelin, RGDCAP) ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human βIG-H3 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human βIG-H3 coated on a 96-well plate. Standards and samples are pipetted into the wells and βIG-H3 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human βIG-H3 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of βIG-H3 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. The minimum detectable dose of βIG-H3 is typically less than 25 pg/ml. This ELISA kit shows no cross-reactivity with the rmβIGH3. The inter assay reproducibility CV is <12% and the intra assay CV is <10%

**II. Sample Type:** Serum, Plasma, Cell culture supernatants and urine.

**III. Kit Contents:**

| Components | K926-100 | Part No. |
|---|---|---|
| βIG-H3 Microplate (Item A): 96 wells coated with anti-human βIG-H3 | 12 stripsx8 wells | K926-100-1 |
| Wash Buffer Concentrate (Item B) (20x) | 25 ml | K926-100-2 |
| Standards (Item C): recombinant human βIG-H3 | 2 vials | K926-100-3 |
| Assay Diluent A (Item D): for Standard/Sample (serum/plasma) diluent. 0.09% sodium azide as preservative | 30 ml | K926-100-4 |
| Assay Diluent B [5X] (Item E): for Standard/Sample (cell culture medium/urine) diluent | 15 ml | K926-100-5 |
| Detection Antibody βIG-H3 (Item F): biotinylated anti-human βIG-H3 (each vial enough for half plate) | 2 vial | K926-100-6 |
| HRP-Streptavidin Concentrate [300X] (Item G) | 200 µl | K926-100-7 |
| TMB One-Step Substrate Reagent (Item H): 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution | 12 ml | K926-100-8 |
| Stop Solution (Item I): 0.2 M sulfuric acid | 8 ml | K926-100-9 |

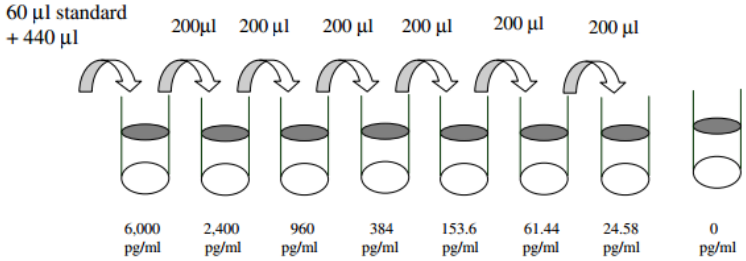
**IV. User Supplied Reagents and Equipment:**

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µl to 1 ml volumes.
- Adjustable 1-25 ml pipettes for reagent preparation.
- 100 ml and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

**V. Storage and Handling:** May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

**VI. Reagent Preparation and Storage Conditions:**

- Bring all reagents and samples to room temperature (18 - 25°C) before use.
- Sample dilution: If your samples need to be diluted, Assay Diluent A (Item D) should be used for dilution of serum/plasma samples.1x Assay Diluent B (Item E) should be used for dilution of culture supernatants and urine. Suggested dilution for normal serum/plasma: 400-4,000 fold. Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.
- Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
- Preparation of standard: Briefly spin the vial of Item C and then add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernates/urine) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 60 µl βIG-H3 standard (50 ng/ml) from the vial of Item C, into a tube with 440 µl Assay Diluent A or 1x Assay Diluent B to prepare a 6,000 pg/ml standard solution. Pipette 300 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the 6,000 pg/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).

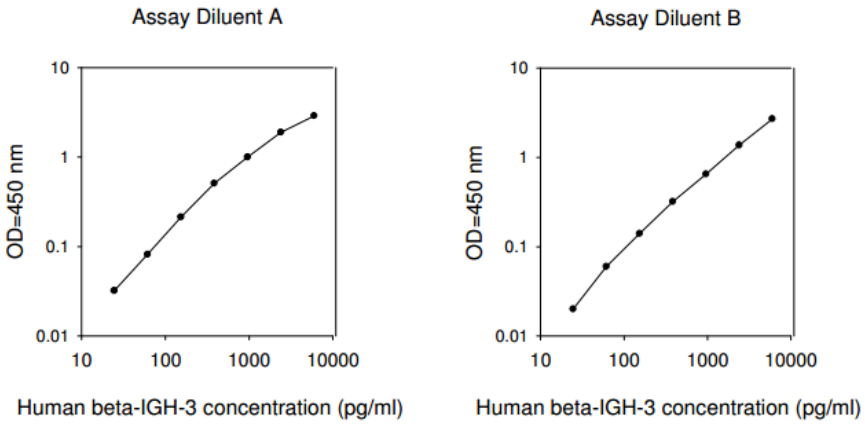



- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
- Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure.

- Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 300-fold with 1x Assay Diluent B. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 50 µl of HRP-Streptavidin concentrate into a tube with 15 ml 1x Assay Diluent B to prepare a 300-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

**VII. Assay Protocol:**

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or O/N at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
5. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.
7. Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.




**VIII. Recovery:** Recovery was determined by spiking various levels of human βIG-H3 into human serum, plasma and cell culture media. Mean recoveries are as in the table:

| Sample type | Average % recovery | Range % |
|---|---|---|
| Serum | 92.44 | 76-113 |
| Plasma | 86.91 | 69-104 |
| Cell culture media | 104.8 | 96-114 |

**IX. Linearity:**

| Sample type | Serum | Plasma | Cell culture media |
|---|---|---|---|
| 1:2 Average % of expected Range (%) | 107.9<br>100-116 | 104.9<br>97-113 | 109.1<br>101-117 |
| 1:4 Average % of expected Range (%) | 116.2<br>108-124 | 113.0<br>95-121 | 78.96<br>70-87 |

**X. RELATED PRODUCTS:**

TGF-β1 Antibody
Recombinant proteins
Apoptosis Assay Kits and Reagents
HDAC and HAT Assay Kits
siRNA Expression Vectors
Polyclonal and monoclonal antibodies
TGF-β1 blocking peptide
Antibodies and ELISA kits Metabolism Assay kits and Reagents
Cellular Fractionation Kits
DNA Damage, SOD Quantification Kits
Recombinant Growth Factors and Cytokines

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