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183170 Beta Defensins (BDF) BioAssay™ ELISA Kit (Chicken)

Specifications

Kit Type
Competitive ELISA

Tests
4896

Sample Volume
100ul

Sensitivity
As reported

Detection Method
Colorimetric

Detection Range
As reported

Sample Matrix
Serum, plasma, cell culture supernatants, body fluid and tissue homogenate.

EU Commodity Code
30021091

UN DOT Shipping
UN2796 PGII

Shipping Temp
Blue Ice

Storage Temp
4°C

The BioAssay™ ELISA Kit is a 1.5 hour solid-phase Competitive ELISA designed for the quantitative determination of Beta Defensins (BDF) (Chicken). This kit for research use only, not for therapeutic or diagnostic applications.

Kit Detection Range

As reported

Kit Sensitivity

As reported

Assay Principle

Beta Defensins (BDF) BioAssay™ ELISA Kit (Chicken) utilizes the quantitative Competitive Enzyme Immunoassay technique, utilizing a monoclonal antibody and an conjugate to Beta Defensins (BDF) (Chicken). The assay sample and buffer are incubated together with the HRP conjugate in a pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the Target Protein concentration since Protein from samples and Protein-HRP conjugate compete for the anti-Protein antibody binding site. Since the number of sites is limited, as more sites are occupied by Protein from the sample, fewer sites are left to bind the Protein-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The Target Protein concentration in each sample is interpolated from this standard curve.

Materials and Equipment Supplied

1. Microtiter Plate, 96 wells, stripwell 2. Enzyme Conjugate, 6mL, 1 vial 3. , 1 vial 4. Standard B, 50pg/mL, 1 vial 5. Standard C, 100pg/mL, 1 vial 6. Standard D, 250pg/mL, 1 vial 7. Standard E, 500pg/mL, 1 vial 8. Standard F, 1000pg/mL, 1 vial 9. Substrate A, 6mL, 1 vial 10. Substrate B, 6mL, 1 vial 11. Stop Solution, 6mL, 1 vial 12. Wash Solution (100X), 10mL, 1 vial 13. Balance Solution, 3mL, 1 vial 14. Protocol Manual

Materials and Equipment Required, but not Supplied

1. Precision pipettors and disposable tips to deliver 10-1000uL. A multi-channel pipette is desirable for large assays. 2. 100mL and 1 Liter graduated cylinders. 3. Distilled or deionized water. 4. Tubes to prepare sample dilutions. 5. Absorbent paper. 6. Microplate reader capable of measuring absorbance at 450nm. 7. Centrifuge capable of 3000×g. 8. Microplate washer or washing bottle. 9. Incubator (37°C). 10. Data analysis and graphing software.

4. Specimen Collection and Storage

Sample Type I: serum or plasma Sample Type II: cell culture supernatants, body fluid and tissue homogenate

Simple Protocol

Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately 1000 × g (or 3000rpm) for 15 minutes. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g (or 3000rpm) at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS

(0.02mol/L, pH 7.0-7.2) to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in a certain amount of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1500×g (or 5000rpm). Remove the supernate and assay immediately or aliquot and store samples at -20°C or -80°C. Cells should be lysed according to the following directions- 1. Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly. 2. Wash cells three times in PBS. 3. Cells were resuspended in PBS and subjected to ultrasonication for 3 times. Alternatively, freeze cells at -20 °C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times. 4. Centrifuge at 1000×g (or 3000rpm) for 15 minutes at 2-8 °C to remove cellular debris. 5. Assay immediately or store samples at -20°C or -80°C. Centrifuge cell culture media at 1000×g (or 3000rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20°C or -80°C.   Please read Reagent Preparation above before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microtiter plate. 1. Secure the desired numbers of coated wells in the holder then add 100uL of Standards or Samples to the appropriate well in the antibody pre-coated Microtiter Plate. Add 100uL of PBS (pH 7.0-7.2) in the blank control well. 2. Dispense 10uL of Balance Solution into 100uL specimens, mix well. Note: This step is required when the sample is cell culture supernatants, body fluid and tissue homogenate; if the sample is serum or plasma, then this step should be skipped. 3. Add 50uL of Conjugate to each well (NOT blank control well). Mix well. Mixing well in this step is important. Cover and incubate the plate for 1 hour at 37°C. 4. Wash the microtiter plate using one of the specified methods indicated (manual or automatic). 5. Add 50uL Substrate A and 50uL Substrate B to each well including blank control well, subsequently. Cover and incubate for 10-15 minutes at 20-25°C. (Avoid sunlight). 6. Add 50uL of Stop Solution to each well including blank control well. Mix well. 7. Determine the Optical Density (O.D.) at 450nm using a microplate reader immediately.

Typical Certificate of Analysis

Important! Standard curve for demonstration only. Please refer to lot-specific Kit Protocol.

1. Same Lot CV%: 4.4, 5.6 2. Different Lot CV%: 6.6, 7.9 3. Spike Recovery: 94-103%

4. Linearity Range % 1:2 96- 101 1:4 93 - 107 1:8 92 - 100 1:16 96 - 108

Contact

5. Sensitivity: 1.0 pg/mL. 6. Specificity: This assay has high sensitivity and excellent specificity for detection of the target antigen. No significant cross-reactivity or interference with analogues was observed. Note: Cross-reactivity detection between the target antigen and all analogues has not been determined and cross-reaction may exist in some cases.

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List Price: $622

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Sizes:

48Tests

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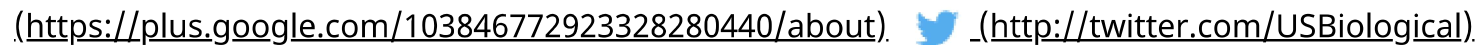
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