Datasheet for ABIN1379938

Defensin beta 3 ELISA Kit

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Overview

Quantity:	96 tests
Target:	Defensin beta 3 (DEFB3)
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The OmniKine™ Human BD-3 ELISA Kit contains the components necessary for quantitative determination of natural or recombinant hBD-3 concentrations within any experimental sample including cell lysates, serum and plasma.
Brand:	OmniKine™
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The Human BD-3 ELISA is capable of recognizing both recombinant and naturally produced Human BD-3 proteins. The antigens listed below were tested at 50 ng/mL and did not exhibit significant cross reactivity or interference. Human: BD-1 (36 aa), BD-1 (47 aa), BD-2
Characteristics:	The Human BD-3 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human BD-3 proteins within the range of 64-4000 pg/mL.
Components:	96-Well Microplate or Strips Coated w/ Capture Antibody: 12 x 8 Strips

Product Details

	Biotin-Conjugated Detection Antibody: Lyophilized, Yellow 50 µL H2O, Use Detection Antibody Diluent Ready-to-Use Avidin-HRP Conjugate Solution: 11 mL, Clear Cytokine Protein Standard: Lyophilized (100 ng), Red 100 µL H2O,Use Protein Standard Diluent Ready-to-Use Substrate: 11 mL, Brown Stop Solution: 11 mL, Clear Adhesive Plate Sealers: 4 Sheets Wash Buffer (10X): 50 mL, Clear,Dilute to 1X Using Pure H2O Protein Standard Diluent: 11 mL, Clear Detection Antibody Diluent: 11 mL, Clear
Material not included:	The following materials and/or equipment are NOT provided in this kit but are necessary to successfully conduct the experiment: Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm) Micropipettes with capability of measuring volumes ranging from 1 µl to 1 ml Deionized or sterile water Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer Graph paper or computer software capable of generating or displaying logarithmic functions Absorbent paper or vacuum aspirator Test tubes or microfuge tubes capable of storing ≥1 ml Bench-top centrifuge (optional) Bench-top vortex (optional) Orbital shaker (optional)

Target Details

Target:	Defensin beta 3 (DEFB3)
Alternative Name:	BD-3 (DEFB3 Products)
Background:	Human BD-3, also known as Beta-Defensin 3, is a 67 amino acid cytokine protein encoded by the DEFB103A gene located at locus 8p23.1 on chromosome 8. After signal sequence processing and maturation, the cytokine becomes 45 amino acid residues long (from 23 to 67). Human BD-3 is known to exhibit antimicrobial activity against Gram-positive bacteria, S.aureus and S.pyogenes, Gram-negative bacteria P.aeruginosa and E.coli and the yeast C.albicans. Moreover, the cytokine kills multiresistant S.aureus and vancomycin-resistant E.faecium. This

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	particular BD protein is expressed highly in skin and tonsils, and to a lesser extent in trachea, uterus, kidney, thymus, adenoid, pharynx and tongue. It has much lower expression in salivary glands, bone marrow, colon, stomach, polyps and larynx along with no expression in the small intestine. Furthermore, induction of BD-3 is achieved through bacterial infection or detection of lipopolysaccharides (LPS) and by IFN-gamma.
UniProt:	P81534
Pathways:	Production of Molecular Mediator of Immune Response

Application Details

Plate:	Pre-coated
Protocol:	This particular immunoassay utilizes the quantitative technique of a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a sandwich format by the primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator. The capture antibodies coated to the bottom of each well are specific for a particular epitope on the Human BD-3 cytokine while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase. After incubation and sandwiching of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3', 5, 5'- Tetramethylbenzidine) is added, the reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration. Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 N Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.
Assay Procedure:	Addition of Known Standard and Unknown Sample to Immunoassay Prior to applying an unknown sample to the Sandwich ELISA, the immunoassay must be performed using a serial dilution of a known standard sample in order to determine the standard curve. This is necessary to allow for the interpretation of results generated by the unknown samples. 1. Dilute the known standard sample from 4 ng/mL to 0 ng/mL in a series of microfuge tubes. Mix each tube thoroughly by inverting several times or by vortexing lightly to ensure proper

equilibration. Add 100 µL of each serial dilution step into the wells of a specified row or column of the 96-well microtiter plate in duplicate or triplicate and incubate at room temperature for 2 hours. Seal the microplate air-tight using one of the microplate adhesive seals provided in this kit or Parafilm if readily available. Note: If a standard curve has already been generated, substitute the standard with the unknown sample of interest.

Application of Detection Antibody to Capture Antibody-Bound Samples
1. Aspirate the protein standard solution out of the microplate wells. If your lab does not have a vacuum-based aspirator, you may dump the solutions from the microplate into a waste container and blot 3-4 times on a stack of paper towels until most or all of the liquid is removed from the wells. Dilute the 10X wash buffer to 1X using pure H2O. Add 300-400 µL of Wash Buffer to each well being used and gently shake for 5-7 minutes on an orbital shaker. Perform this wash step 4 times consecutively.
2. After the 4th wash step, dilute the detection antibody solution 1:400 in detection antibody diluent to a concentration of 0.25 µg/mL. Mix the test tube either by inverting several times or vortexing to ensure proper equilibration. Ensure that there is enough detection antibody solution for all wells being used. Add 100 µL of the diluted detection antibody solution into each well, seal the plate and incubate at room temperature for 2 hours.

Conjugation of Avidin-Horseradish Peroxidase Enzyme with Detection Antibody
1. Remove the detection antibody solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Perform 4 consecutive wash steps with gentle shaking between each wash.
2. After the 4th wash step, add 100 µL of Ready-to-Use Avidin-HRP Conjugate Solution into each well and incubate at room temperature for 30 minutes.

Application of Liquid Substrate for Colorimetric Reaction
1. Remove the Avidin-HRP conjugate solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Prepare the TMB substrate solution by bringing it to room temperature without exposure to fluorescent or UV light as these may degrade the TMB. Perform 4 consecutive wash steps with gentle shaking between each wash.
2. After the 4th wash step, add 100 µL of TMB substrate solution into each well and incubate at room temperature for color development. The microplate should be kept out of direct light by either covering with an opaque object or putting it into a dark room. Closely monitor the color development as some wells may turn blue very quickly depending on analyte and/or detection antibody-HRP concentrations. Once the blue color has ceased to develop further, immediately

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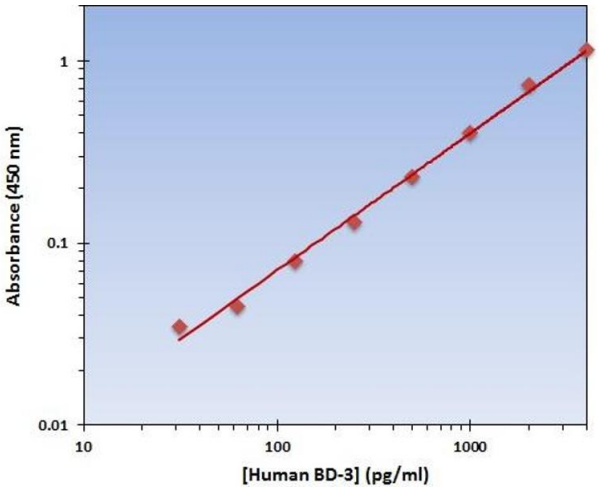
	add 100 µL of Stop Solution to each well being used. The color in the wells should immediately change from blue to yellow. 3. The microplate is now ready to be read by a microplate reader. Within 30 minutes of adding the Stop Solution, determine the optical density (absorbance) of each well by reading the plate with the microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. Caution: Readings made directly at 450 nm without correction may be higher and less accurate.
Calculation of Results:	Generation of Standard Curve and Interpretation of Data 1. Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density. 2. Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4- Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis). Note: Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or trend-line through the plotted points via regression analysis.
Restrictions:	For Research Use only

Handling

Precaution of Use:	Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment. Stop Solution contains 2 N Sulfuric Acid (H2SO4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.
Storage:	4 °C/-20 °C
Storage Comment:	If used frequently, reagents may be stored at 2-8°C. If used infrequently, reagents should be stored at -20°C.



ELISA

Image 1. This is an example of what a typical standard curve will look like. You must make your own standard curve. Do not use this example as your own standard curve.