# antibodies - online.com







# **DEFa3 ELISA Kit**







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Quantity:	96 tests
Target:	DEFa3
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.47 ng/mL - 30 ng/mL
Minimum Detection Limit:	0.47 ng/mL
Application:	ELISA
Product Details	
Purpose:	The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of DEFa3 in Serum,Plasma,Biological Fluids
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Defensin Alpha 3, Neutrophil Specific (DEFa3).  No significant cross-reactivity or interference between Defensin Alpha 3, Neutrophil Specific (DEFa3) and analogues was observed.
Cross-Reactivity (Details):	No significant cross-reactivity or interference between Defensin Alpha 3, Neutrophil Specific (DEFa3) and analogues was observed.
Sensitivity:	0.21 ng/mL

### **Product Details**

#### Components:

- · Pre-coated, ready to use 96-well strip plate, flat buttom
- · Plate sealer for 96 wells
- · Reference Standard
- · Standard Diluent
- · Detection Reagent A
- · Detection Reagent B
- · Assay Diluent A
- · Assay Diluent B
- Reagent Diluent (if Detection Reagent is lyophilized)
- · TMB Substrate
- · Stop Solution
- Wash Buffer (30 x concentrate)
- · Instruction manual

# **Target Details**

Target:	DEFa3
Alternative Name:	DEFa3 (DEFa3 Products)
UniProt:	P59666
Pathways:	Cellular Response to Molecule of Bacterial Origin

# **Application Details**

# Application Notes:

- Limited by the current condition and scientific technology, we cannot completely conduct the
  comprehensive identification and analysis on the raw material provided by suppliers. So
  there might be some qualitative and technical risks to use the kit.
- The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
- Kits from different batches may be a little different in detection range, sensitivity and color developing time.
- Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
- Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
- There may be some foggy substance in the wells when the plate is opened at the first time. It
  will not have any effect on the final assay results. Do not remove microtiter plate from the
  storage bag until needed.
- Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader

with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at  $450 \pm 10$ nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.

- Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
- Each kit has been strictly passed Q.C test. However, results from end users might be
  inconsistent with our in-house data due to some unexpected transportation conditions or
  different lab equipments. Intra-assay variance among kits from different batches might arise
  from above factors, too.
- Kits from different manufacturers for the same item might produce different results, since
  we have not compared our products with other manufacturers.

#### Comment:

Information on standard material:

The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative.

Information on reagents:

The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them.

Information on antibodies:

The provided antibodies and their host vary in different kits.

Sample Volume:

100 μL

Assay Time:

3 h

Plate:

Pre-coated

Protocol:

The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Defensin Alpha 3, Neutrophil Specific (DEFa3). Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to Defensin Alpha 3, Neutrophil Specific (DEFa3). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Defensin Alpha 3, Neutrophil Specific (DEFa3), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured

spectrophotometrically at a wavelength of  $450 \, \text{nm} \pm 10 \, \text{nm}$ . The concentration of Defensin Alpha 3, Neutrophil Specific (DEFa3) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

### Reagent Preparation:

- 1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.
- 2. Standard Reconstitute the Standard with 1.0 mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 60 ng/mL. Firstly dilute the stock solution to 30 ng/mL and the diluted standard serves as the highest standard (30 ng/mL). Then prepare 7 tubes containing 0.5 mL Standard Diluent and use the diluted standard to produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 30 ng/mL, 15 ng/mL, 7.5 ng/mL, 3.75 ng/mL, 1.88 ng/mL, 0.94 ng/mL, 0.47 ng/mL, and the last microcentrifuge tube with Standard Diluent is the blank as 0 ng/mL.
- 3. Detection Reagent A and Detection Reagent B If lyophilized reconstitute the Detection Reagent A with 150µL of Reagent Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
- 4. Wash Solution Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).
- 5. TMB substrate Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

#### Note:

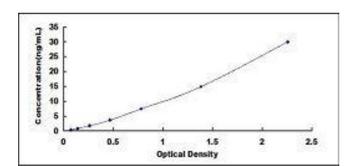
- 1. Making serial dilution in the wells directly is not permitted.
- 2. Prepare standards within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
- 3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for one pipetting.
- 4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
- 5. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
- 6. Contaminated water or container for reagent preparation will influence the detection result.

# Assay Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Defensin Alpha 3, Neutrophil Specific (DEFa3) were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Defensin Alpha 3, Neutrophil Specific (DEFa3) were tested on 3 different plates, 8 replicates in

# **Application Details**

	each plate.
	CV(%) = SD/meanX100
	Intra-Assay: CV<10%
	Inter-Assay: CV<12%
Restrictions:	For Research Use only
Handling	
Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and
	clothing protection when using this material.
Handling Advice:	The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than
	5 % within the expiration date under appropriate storage condition.
	To minimize extra influence on the performance, operation procedures and lab conditions,
	especially room temperature, air humidity, incubator temperature should be strictly controlled. I
	is also strongly suggested that the whole assay is performed by the same operator from the
	beginning to the end.
Storage:	4 °C
Storage Comment:	<ul> <li>For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C upon receipt while the others should be at 4°C.</li> </ul>
	For opened kit: When the kit is opened, the remaining reagents still need to be stored
	according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.
	Note: It is highly recommended to use the remaining reagents within 1 month provided this is
	within the expiration date of the kit.
	<ul> <li>For ELISA kit, 1 day storage at 37°C can be considered as 2 months at 4°C, which means 3 days at 37°C equaling 6 months at 4°C.</li> </ul>
Expiry Date:	6 months



# **ELISA**

Image 1.





### Successfully validated (ELISA (ELISA))

by Shakti Bioresearch

Report Number: 029584

Date: Jan 22 2014

Lot Number:	L131225503
Method validated:	ELISA (ELISA)
Positive Control:	Human serum
Negative Control:	Mouse serum
Notes:	Minimal levels of signal detected in negative control. Percent recovery of the spiked samples shows that there is matrix interference. Dilution of >50 fold is required for accurate measurement of the analyte in human serum samples.
Primary Antibody:	- Antigen: Defensin, alpha 3, Neutrophil-Specific (DEFa3) - Catalog number: ABIN419782 - Supplier: USCNK - Supplier catalog number: SEE135Hu - Lot number: L131225503
Controls:	<ul> <li>Positive control: normal human serum</li> <li>Negative control: mouse serum</li> <li>Standard curve: serial two-fold dilutions from 30 ng/ml [30, 15, 7.5, 3.75, 1.875, 0.9375, 0] were generated from the standard provided in the kit using standard diluent buffer.</li> <li>Spike control: standard diluted in human or mouse serum [6 ng/mL].</li> <li>Control lyophilized powder was dissolved in 150uL standard diluent buffer (7.39 ng/mL)</li> </ul>
Protocol:	<ul> <li>All reagents in the ELISA kit were brought up to room temperature (RT) before use.</li> <li>100 µl of each sample was added per well to the micro ELISA plate well. All samples and standards were assayed in triplicate.</li> <li>The plate was covered with sealer (provided in kit) and incubated for 120 mins at 37°C.</li> </ul>

- · Liquid was removed from each well by pipette.
- Detection Reagent A was diluted 100 fold in Assay Diluent A. 100 µl of diluted detection reagent A was added to each well and the plate was sealed. The plate was tapped to ensure mixing and incubated for 60 mins at 37°C.
- Wells were washed with 300 µl wash buffer three times. Each wash involved fully aspirating the liquid from each well by pipette. After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.
- Detection Reagent B was diluted 100 fold in Assay Diluent B. 100 µl of diluted detection reagent B was added to each well and the plate was sealed. The plate was tapped to ensure mixing and incubated for 30 mins at 37°C.
- Wells were washed with 300  $\mu$ l wash buffer five times. Each wash involved fully aspirating

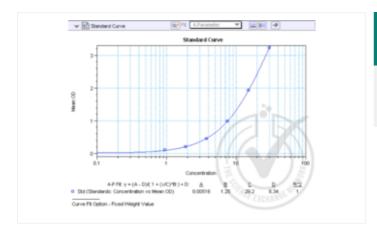


- the liquid from each well by pipette. After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.
- 90 µl of Substrate Solution was added to each well and the plate was covered with a new plate sealer. The plate was tapped to ensure mixing and incubated at room temperature in the dark.
- After about 10 mins, when an apparent gradient appeared in the standard wells, the reaction was terminated by adding 50 µl of Stop Solution to each well.
- The optical density (OD value) of each well was read using a micro-plate reader set to 450
- · The triplicate readings for each sample were averaged and the average zero standard optical density subtracted. A standard curve was generated by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis using Softmax Pro softare.
- The equation  $y = (A-D)/(1 + (x/C)^A) + D$  was used to calculate IL-6 concentrations of the samples based on their average OD values.

#### **Experimental Notes:**

- Percent recovery of the spiked samples shows that there is matrix interference. Dilution of >50 fold is required for accurate measurement of the analyte in human serum samples.

# Images for Validation report #029584



# Validation image no. 1 for Defensin, alpha 3, Neutrophil-Specific (DEFa3) ELISA Kit (ABIN419782)

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Sample Description	00 450nm	Conc (ng/ mL)	Mean Cono (ng/mL)	Std. Dev.	CV%	Dilution Factor	Conc (ng/mL) in neat serum	% Recovery
	2.482	20.48		2.79	16.2	10	172.59	
Human serum	2.004	15.72	17.26					
diluted 10 fold	1.989	15.58						
6 ng/mL of	2.532	21.03		2.15	10.8			
DEFa3 spiked	2.554	21.28	19.91					85.6
into diluted human serum	2.185	17.43						
	0.790	6.11		0.71	12.9	50	275.55	
Human serum diluted 50 fold	0.732	5.70	5.51					
	0.594	4.73					0	
6 ng/mL of DEFa3 spiked	1.237	9.37	8.62	0.68	7.9		-0"	
	1.115	8.46						74.9
into diluted human serum	1.058	8.04						
	0.060	0.66	0.56	0.08	198	19	5.63	
Mouse serum	0.046	0.52						
diluted 10 fold	0.045	0.51						/ / I
Mouse serum	0.028	0.33		0.03	7.9	50	16.35	
diluted 50 fold	0.030	0.35	0.33					
GHUNDO DU TORO	0.026	0.30						1 %
100 µL of Control sample tested	0.423	3.51	3.51		1.	6	35.08	474.7
30 µL of Control sample tested	0.171	1.62	1.62			Mer	53.93	729.8
15 µL of Control sample tested	0.100	1.03	1.03				68.80	931.0

# Validation image no. 2 for Defensin, alpha 3, Neutrophil-Specific (DEFa3) ELISA Kit (ABIN419782)

Table 1: ELISA. DEFa3 could be detected in human serum (positive control) after 10 fold dilution. Spike controls indicate that there is interference from the human serum matrix and a dilution of >50 fold is required. Mouse serum was used as negative control, there were residual levels of DEFa3. Control sample concentration is expected to be 7.39

ng/mL

Sample	Concentration (ng/mL)	BackCalcConc (ng/mL)	OD 450nm	Mean OD	Std.Dev.	CV%	%Accuracy
St02		30.41	3.254	3.227	0.068	2.1	101.4
	30	30.76	3.277				102.5
		28.85	3.15				96.2
		14.46	1.864	1.927	0.083	4.3	96.4
St03	15	15.88	2.022				105.8
		14.73	1.895				98.2
St04		8.43	1.11	0.984		11.2	112.3
	7.5	7.19	0.94		0.111		95.8
		6.92	0.903				92.2
		4.28	0.531	0.448	0.078	17.3	114.1
St05	3.75	3.61	0.437				96.2
		3.18	0.377				84.7
		1.94	0.212	0.203	0.023	11.1	103.7
St06	1.875	2.01	0.22				107.0
		1.67	0.178				89.3
	0.938	1.20	0.12	0.106	0.014	13.1	127.9
St07		1.08	0.106				115.6
		0.96	0.092				102.0
St08	0	Range?	-0.001	-0.005	0.003	68.2	15.00
		Range?	-0.005				
		Range?	-0.008				-

# Validation image no. 3 for Defensin, alpha 3, Neutrophil-Specific (DEFa3) ELISA Kit (ABIN419782)

Table 2: Value for Average Reading is derived from the average of three readings (OD 450nm). The Average Reading for BLANK (0 ng/ml) was subtracted from all Average Readings to yield Average Absorbance values for Standards. Standard deviation is included for all samples. An equation  $y = (A-D)/(1 + (x/C)^A) + D$  was generated from the standard curve and used to calculate DEFa3 concentrations shown in the Figure 1.