ELISA Kit Development Service

EGFR

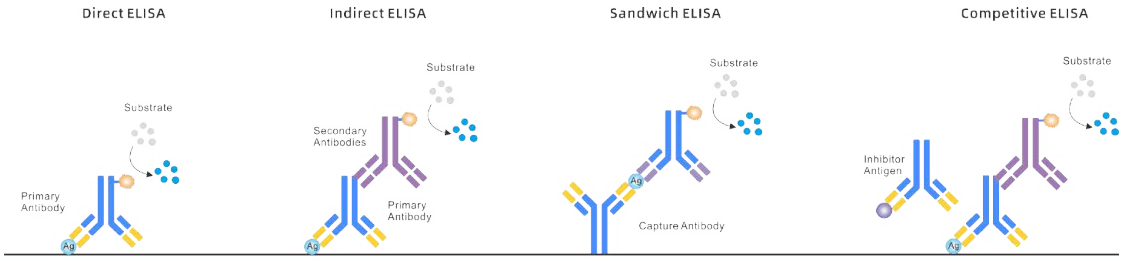
Enzyme Linked Immunosorbent Assay (ELISA) is known as absorbing antigens or antibodies on the surface of solid phase and forming immune complexes through the specific reaction of antigen-antibody-enzyme labeled antibodies. The enzyme catalyzes the substrate to produce color reaction for qualitative or quantitative analysis of antigen or antibody. ELISA method is the most widely used technique in immunoassay. It has the characteristics of high throughput detection, simple operation, strong specificity and high sensitivity. At present, the commonly used ELISA methods include direct method, indirect method, sandwich method and competitive ELISA.

Based on antigen-antibody interaction, ELISA kit is widely used in food safety, disease diagnosis, antibody drug development and other fields, and is an important research and diagnostic tool.

AtaGenix has a complete antigen and antibody research and development platform, professional technical team and rich experience in detection. We can provide customers with one-stop ELISA kit development services: antigen design and expression, antibody preparation, antigen/antibody labeling, antibody pair screening, ELISA kit experimental conditions optimization, ELISA kit assembly and production, ELISA kit methodology evaluation, etc.

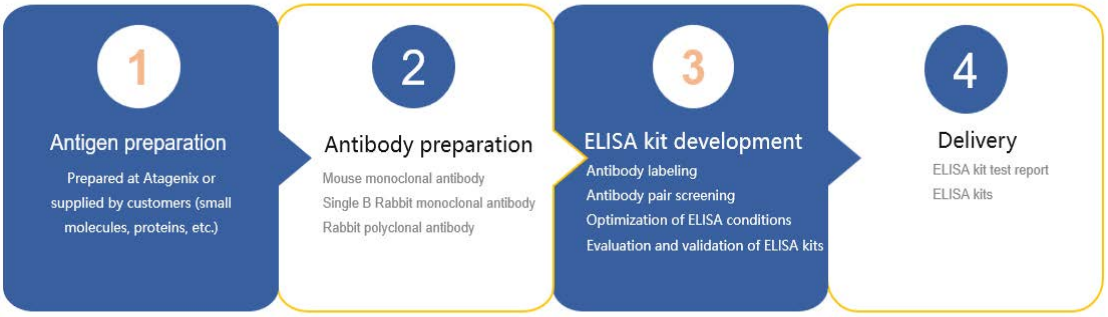
Advantage of service

Service Type



Standard ELISA Kits

EGFR



ELISA kit development service details

Step	Service content	Lead Time	Deliverables
ELISA kit development	Screening the best antibody pairs ELISA condition optimization Standard curve Natural sample assay	2~3 weeks	Data report
ELISA kit Performance evaluation	Accuracy (recovery measurement) Dilution linearity Sensitivity (MDD measurement) Precision (Intra-Assay and Inter-Assay CV measurement) Stability	3-4 weeks	Data report
ELISA Kit production	ELISA kit production	2-3 weeks	COA

Sandwich ELISA Kit Experimental process

Bring all reagents and samples to room temperature before use.

1. Dilute the Coating Antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100 µL per well of the diluted Coating Antibody. Seal the plate and incubate overnight at 4℃ .

2. Aspirate each well and invert the plate and blotting it against clean paper towels. Block plates by adding 300 µL of 3%BSA to each well. Incubate for 1.5 hour at 37℃ .

3. Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or auto-washer. 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 100µL of standard, control, or samples per well. Cover with the adhesive strip provided. Incubate for 1 hour at 37℃ .

5. Repeat the aspiration/wash as in step 3.

6. Add 100µL of Detection antibody (working solution) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37℃ .

7. Repeat the aspiration/wash as in step 3.

8. Add 100µL of Streptavidin-HRP (working solution) to each well. Cover with a new adhesive strip. Incubate for 0.5 hour at 37℃ .

9. Repeat the aspiration/wash as in step 3.

10. Add 100µL of Color Reagent to each well. Incubate for 10 minutes at room temperature. Protect from light.

11. Add 50µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

12. Determine the optical density of each well within 10 minutes, using a 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. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Bring all reagents and samples to room temperature before use.

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).Create a standard curve by reducing the data using computer software capable. Construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the predecessor protein concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

AtaGenix recommends 4-PL four-parameter fitting, which you can do with software such as ELISACalc, Origin, etc., which is suitable for most ELISA standard fits.

ELISA kit development

Checkerboard screening

All antibodies were labeled and pairwise matched with other antibodies by checkerboard method to screen out the best combination of antibody pairs.

Detection Ab/Capture Ab	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10
Biotin-Ab1	/	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Ag
Biotin-Ab2	Ag	/	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Ag
Biotin-Ab3	Ag	Ag	/	Ag	Ag	Ag	Ag	Ag	Ag	Ag
Biotin-Ab4	Ag	Ag	Ag	/	Ag	Ag	Ag	Ag	Ag	Ag
Biotin-Ab5	Ag	Ag	Ag	Ag	/	Ag	Ag	Ag	Ag	Ag
Biotin-Ab6	Ag	Ag	Ag	Ag	Ag	/	Ag	Ag	Ag	Ag
Biotin-Ab6	Ag	Ag	Ag	Ag	Ag	Ag	/	Ag	Ag	Ag
Biotin-Ab8	Ag	Ag	Ag	Ag	Ag	Ag	Ag	/	Ag	Ag
Biotin-Ab9	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Ag	/	Ag
Biotin-Ab10	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Ag	Ag	/

ELISA condition optimization

The capturer antibody and the detection antibody are diluted at different concentrations to obtain the optimal working concentration of the antibody pair.

capturer antibody	Coating concentration 1			Coating concentration 2			Coating concentration 3		
Detection antibody	C (Dilution 1)	C (Dilution 2)	C (Dilution 3)	C (Dilution 1)	C (Dilution 2)	C (Dilution 3)	C (Dilution 1)	C (Dilution 2)	C (Dilution 3)
Antigen	Dilution 1								
	Dilution 2								
	Dilution 3								
	Dilution 4								
	Dilution 5								
	Dilution 6								
	Dilution 7								
Blank	Blank								

Natural sample assay

Natural sample testing is to ensure that the kit can detect natural samples and to determine the appropriate type of natural sample. Select the appropriate sample (serum, saliva, urine or milk, etc.) for testing according to the different targets.

ELISA kit Performance evaluation

Accuracy

Accuracy is the degree to which the result measured by the established method is close to the true value, which is assessed by the recovery rate (%).The recovery rate between 80-120% was the ideal result.

Dilution linearity

The linear assay evaluates the matrix effects and determines the accuracy and specificity of the kit. In order to evaluate the linearity of the test, the sample containing a high concentration of the test substance or adding a high concentration of the test substance is continuously diluted with a standard diluent so that the diluted test value is within the standard curve range.

Precision

Precision is the degree of consistency between independent measurement results under specified conditions, evaluated by the coefficient of variation CV%, including Intra-Assay Precision and Inter-Assay Precision, indicating the uniformity within the plate and the uniformity between the plates respectively.

Sensitivity

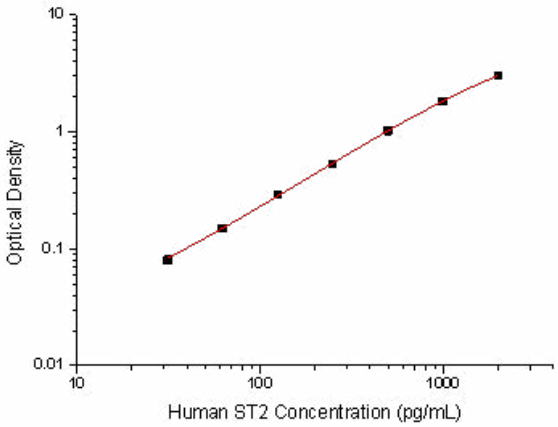
Sensitivity is the ability to detect the lowest concentration of a target in a sample, as assessed by the Minimum detectable dose (MDD).

Stability

Stability test mainly verified the long-term preservation stability of ELISA kit by 37℃ accelerated experiment. Generally, the kit components are placed at 37°C for 3 days equivalent to 4°C for half a year.

Case1 Human ST2 ELISA kit

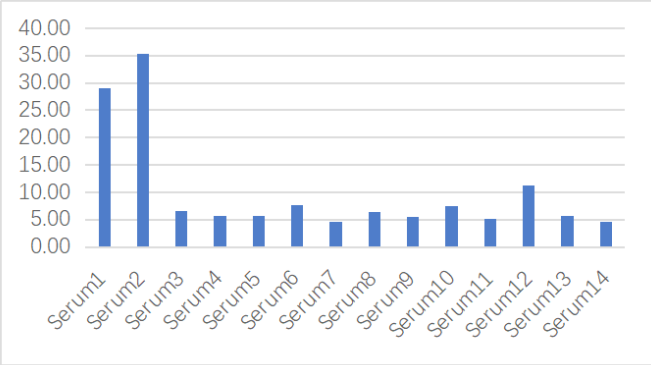
(1) Standard curve and linear range



(pg/mL)	O.D.	Average	Corrected
0	0.02	0.02	-
	0.02		
31.25	0.1	0.1	0.08
	0.1		
62.5	0.17	0.17	0.15
	0.17		
125	0.31	0.31	0.29
	0.31		
250	0.54	0.55	0.53
	0.55		
500	1.03	1.04	1.02
	1.04		
1000	1.81	1.85	1.83
	1.88		
2000	3.04	3.05	3.03
	3.05		

(2) Natural sample assay

Serum or plasma samples from 14 healthy individuals were tested to eliminate individual differences.



(3) Accuracy

Prepare a sample with known concentration, measure it for 3 times, and calculate the ratio of the measured concentration to the theoretical concentration, that is, the recovery rate, in order to test the accuracy of the method.

Sample Type	Average (%)	Range (%)
Cell Culture Supernate (n=3)	112	105-118
Human serum (n=3)	109	103-117

(4) Dilution linearity

The high concentration of antigen was added to the matrix, and the diluent of the standard product was diluted in sequence according to the double gradient, so that the detected value after dilution was within the standard curve range.

Sample Type	Dilution factor	Average (%)	Range (%)
Human serum (n=4)	1:02	98	91-104
	1:04	98	86-105
	1:08	95	86-100
	1:16	93	78-107
Cell Culture Supernate (n=2)	1:02	100	-
	1:04	92	87-100
	1:08	92	88-98
	1:16	90	84-97

(5) Precision

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested sixteen times on one plate to assess intra-assay precision, generally <10%.

Inter-Assay Precision (Precision between assays): Three samples of known concentration were tested in twenty four separate assays to assess inter-assay precision,,generally <15%.

	Intra-batch CV value			Inter-batch CV value		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/mL)	949.7	242.2	60.8	978.8	237.3	57.3
Standard deviation	53.6	11.4	5	40.1	13.7	5.7
CV (%)	5.6	4.7	8.3	4.1	5.8	9.9

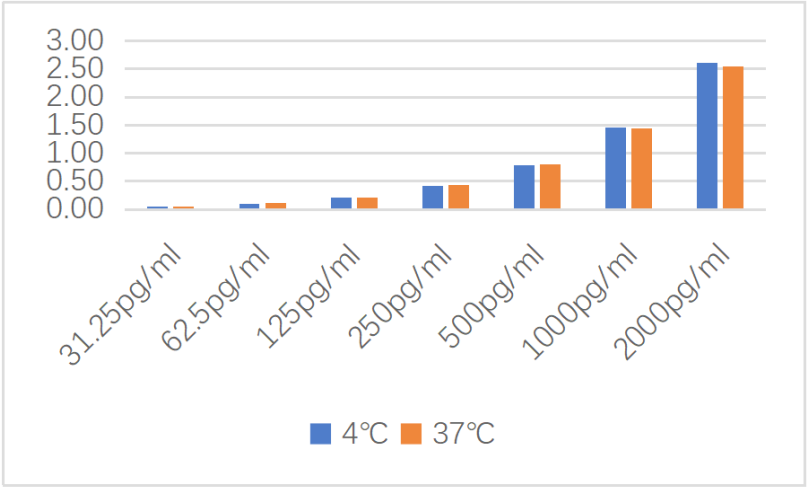
(6) Sensitivity

The MDD is determined by adding 2 standard deviations to the average OD value of 20 standard zero replicates and calculating the corresponding concentration.

MDD=21.22 pg/mL.

(7) Stability

The signal of Human ST2 ELISA kit accelerated at 37℃ for 3 days showed no significant change compared with that of the kit stored at 4℃ , which confirmed that the kit had good stability and could be stored at 4℃ for half a year.



ELISA FAQ

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Bubbles in the wells	Tap plate gently to disperse bubbles
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
High background	Plate is not washed properly	Make sure the wash apparatus works properly
	Incorrect incubation times and/or temperatures	The OD value increased gradually along with the time. Reduce the color developing time properly
Weak/No Signal	Pipetting errors	Make sure the pipette is calibrated
	The working solution not be prepared immediately before use	The working solution should be prepared immediately before use and should not be stored
	Volumes errors	Repeat assay with the required volumes in manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat assay
	Detection A working solution is not completely mixed with the samples	After adding the Detection A into the wells, make sure the detection A and the samples are mixed thoroughly

Chemiluminescent immunoassay

Chemiluminescent immunoassay (CLIA) is a method that combines a chemiluminescent system and an immune reaction to detect the substance to be measured by a chemical reaction that produces photons whose strength is proportional to the number of labeled compounds present. The intensity of light is measured in relative light units (RLU). Chemiluminescence technology has the advantages of high sensitivity, good precision, wide linear range, fast analysis, easy to realize automation, etc., and has been widely used in clinical detection, food safety, life science and other fields. AtaGenix also can provide chemiluminescent ELISA kit development services.

Case2 Interleukin 6 (IL6) Diagnostic Antigen Development and Sandwich Antibody Pair Development

Interleukin 6 (IL6) Diagnostic Antigen Development and Sandwich Antibody Pair Development

IL6 Antigen - expressed by mammalian expression system, high activity, and good stability.

（1）Activity Study:

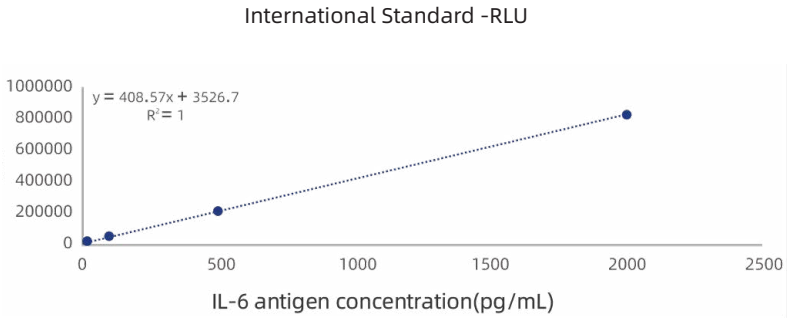
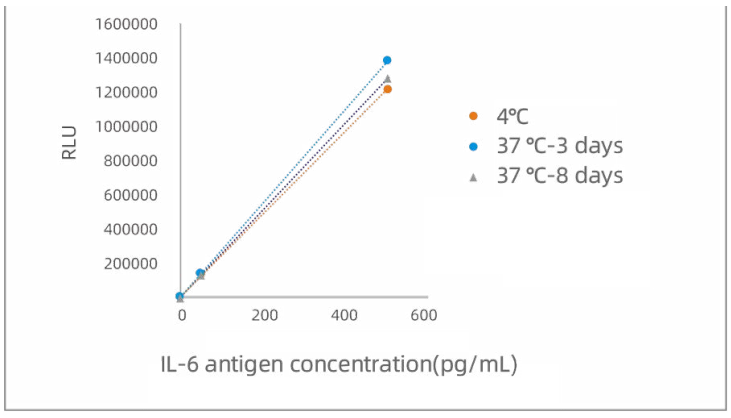


Table 1. Comparison of AtaGenix IL6 with the International Standard

AtaGenix IL6 Antigen (pg/ml)	RLU Mean	Converted concentration (pg/ml) (Corresponding to IL6 international standard concentration)	Ratio	Ratio Mean
5	15603	29.56	5.91	6.06
50	130250	310.16	6.20	

AtaGenix IL6 antigen activity is higher, 1pg/ml IL6 antigen and 6pg/ml international standard reactivity are equivalent. (IL6 International Standard: NIBSC 1st IL6 89/548)

（2）IL6 Antigen Stability Study:



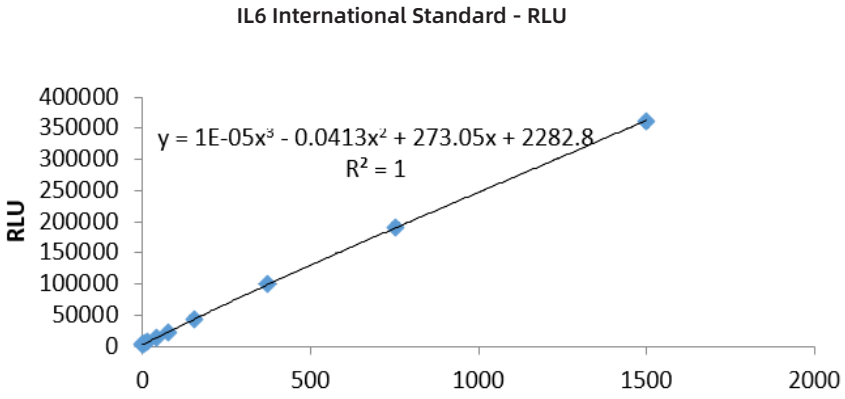
AtaGenix IL6 antigen shows a good stability, with no distinctive differences in RLU values between 3 days at 37°C and 8 days at 37°C and long-term storage at 4°C, and still maintains high activity.

Rabbit Immune Antigen Preparation: Protein expression in proprietary mammalian system.

Screening Antigen: WHO international standard of NIBSC (IL-6, human rDNA derived, NIBSC code 89/548)

Monoclonal Antibody Preparation: A total of 44 IL6-positive cell lines are screened by using mouse hybridoma technology.

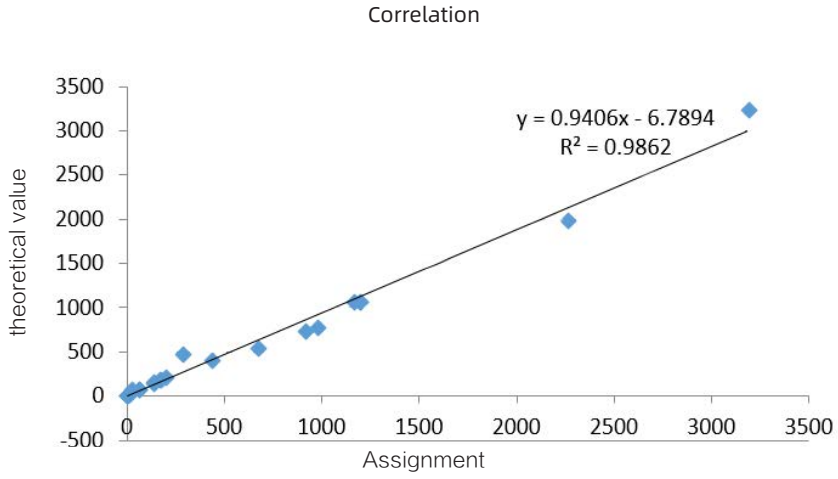
A total of 1892 antibody pairs are screened by the chemiluminescence platform.



In the chemiluminescence platform, Roche measured samples are used for screening. One pair of antibodies is obtained with desirable specificity for serum samples and international standards. The sensitivity is 1pg/ml, and R^2=1.

Parallel Comparison:

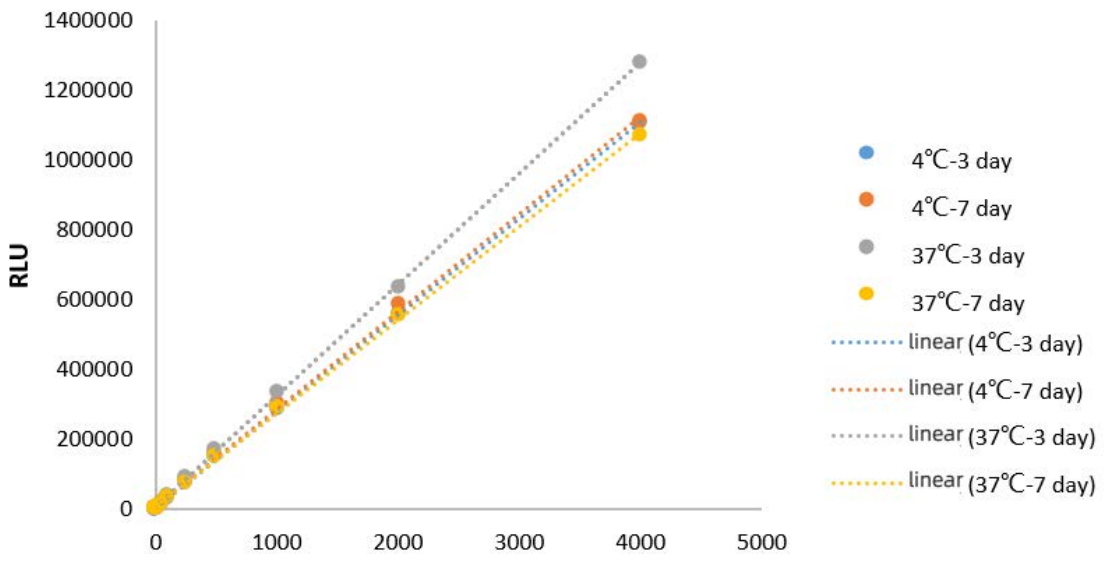
Comparing the theoretical value from Roche, the correlation with the result from AtaGenix is R^2=0.9862.



Stability Test:

AtaGenix IL6 Antibody (pg/ml)	4℃ -3 day-RLU	4℃ -7 day-RLU	37℃ -3 day-RLU	37℃ -7 day-RLU
0	1886	3162	2790	2972
1	2022	3341	3172	3504
2.5	2586	3948	3775	3985
5	3442	4924	4815	4932
10	5110	6539	6483	6569
25	9541	11216	11938	11049
50	17791	19547	20801	18950
100	32047	36028	37825	33702
250	76150	83036	90499	78330
500	150229	157355	171096	149662
1000	289376	305072	336157	291227
2000	560092	587798	636806	553922
4000	1106263	1112355	1280067	1070342

The working solution was placed at 4°C and 37°C for 3 days and 7 days, respectively before test. The results show that the IL6 antibody was very stable.



AtaGenix IL6 antibody stored at 37°C for 3 days/7 days and 4°C for 3 days/7 days - RLU Comparison