Datasheet for ABIN365163

IL-6 ELISA Kit

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Overview

Quantity:	96 tests
Target:	IL-6 (IL6)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	7.8-500 pg/mL
Minimum Detection Limit:	7.8 pg/mL
Application:	ELISA

Product Details

Purpose:	For the quantitative determination of human interleukin 6 (IL-6) concentrations in serum, plasma, cell culture supernates, tissue homogenates and urine.
Sample Type:	Serum, Plasma, Cell Culture Supernatant, Tissue Homogenate, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of human IL-6.
Cross-Reactivity (Details):	Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between the target antigen and all analogues for other species. Therefore, cross reaction may still exist.
Sensitivity:	2.453 pg/mL
Components:	• Assay plate (12 × 8 coated Microwells)

Product Details

- Standard (freeze dried)
- Biotin-antibody (100 × concentrate)
- HRP-avidin (100 × concentrate)
- Biotin-antibody Diluent
- HRP-avidin Diluent
- Sample Diluent
- Wash Buffer (25 × concentrate)
- TMB Substrate
- Stop Solution
- Adhesive Strip (for 96 wells)
- Instruction manual

Target Details

Target:	IL-6 (IL6)
Alternative Name:	Interleukin 6 (IL-6) (IL6 Products)
Background:	Synonyms: BSF2, HGF, HSF, IFNB2, IL-6, B cell stimulatory factor-2|B-cell differentiation factor|CTL differentiation factor|OTTHUMP00000158544|hybridoma growth factor|interleukin 6|interleukin BSF-2
HGNC:	1834
UniProt:	P05231
Pathways:	TLR Signaling, Hormone Transport, Negative Regulation of Hormone Secretion, Myometrial Relaxation and Contraction, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, Regulation of Carbohydrate Metabolic Process, Autophagy, Cell RedoxHomeostasis, Cancer Immune Checkpoints, Inflammasome

Application Details

Application Notes:

- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
- Grossly hemolyzed samples are not suitable for use in this assay.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular

	experiments. • Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals. • Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products. • Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit. • Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
Comment:	Detection wavelength: 450 nm Information on standard material: Depending on the antigen to be detected, standards can be either native or recombinant protein. The recombinant proteins are being expressed in CHO cells in most cases. Please inquire for more information. The formulation of auxiliary material in the standard is considered proprietary information, however it does not contain any poisonous substance. Proclin 300 (1:3000) is used as preservative. Information on reagents: In most cases the stop solution provided is 1 N H2SO4. The formulation of wash solution is proprietary information. None of the components contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials. For the sandwich method kits, the sample diluent, antibody diluent, enzyme diluent and standard all contain BSA. Information on antibodies: The antibodies provided in different kits vary in regards to clonality and host. Some antibodies are affinity purified, some are Protein A
Sample Volume:	100 µL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After removing any

	unbound substances, a biotin-conjugated antibody specific for IL-6 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	• **Biotin-antibody (1×)** - Centrifuge the vial before opening. Biotin-antibody requires a 100-fold dilution. The suggested dilution is 10µL of Biotin-antibody + 990µL of Biotin-antibody Diluent. • **HRP-avidin (1×)** - Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. The suggested dilution is 10µL of HRP-avidin + 990µL of HRP-avidin Diluent. • **Wash Buffer (1×)** - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500mL of Wash Buffer (1×). • **Standard** - Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard. Sample Diluent serves as the zero standard (0ng/mL). Note: • Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit. • Bring all reagents to room temperature (18-25°C) before use for 30 min. • Prepare fresh standard for each assay. Use within 4 hours and discard after use. • Making serial dilution in the wells directly is not permitted. • Please carefully reconstitute Standards according to the instruction. Avoid foaming and mix gently until the crystals have 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 when pipetting. • It is recommended to use distilled water to prepare reagents and samples. Using contaminated water or container for reagent preparation will influence detection result.
Assay Precision:	Intra-assay precision (precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess precision. Inter-assay precision (precision between assays): Three samples of known concentration were tested in twenty assays to assess precision. • Intra-assay: CV% less than 8%

Application Details

	• Inter-assay: CV% less than 10%
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.
Handling Advice:	• The kit should not be used beyond the expiration date on the kit label. • Do not mix or substitute reagents with those from other lots or sources. • If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay. • Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time/temperature and kit age can cause variation in binding. • This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials.
Expiry Date:	6 months

Publications

Product cited in:	Yang, Yu, Dong, Zhang, Du, Zhu, Che, Wang, Shen, Jiang: "Serum macrophage migration inhibitory factor concentrations correlate with prognosis of traumatic brain injury." in: **Clinica chimica acta; international journal of clinical chemistry**, Vol. 469, pp. 99-104, (2018) (PubMed). Yu, Huang, Po, Tan, Wang, Zhou, Meng, Yuan, Zhou, Li, Wang, Wang, Jiang: "Low-Level Tragus Stimulation for the Treatment of Ischemia and Reperfusion Injury in Patients With ST-Segment Elevation Myocardial Infarction: A Proof-of-Concept Study." in: **JACC. Cardiovascular interventions**, Vol. 10, Issue 15, pp. 1511-1520, (2018) (PubMed). Chen, Cheng, Shao, Shentu, Fu: "Macrophage migration inhibitory factor as a serum prognostic marker in patients with aneurysmal subarachnoid hemorrhage." in: **Clinica chimica acta; international journal of clinical chemistry**, Vol. 473, pp. 60-64, (2018) (PubMed).

Publications

Wang, Yan, Peng, Jiang, He, Peng, Chen, Ye, Zhuo: "Functional Role of SUV39H1 in Human Renal Tubular Epithelial Cells Under High-glucose Ambiance." in: **Inflammation**, Vol. 41, Issue 1, pp. 1-10, (2018) (PubMed).

Golkhalkhali, Rajandram, Paliany, Ho, Wan Ishak, Johari, Chin: "Strain-specific probiotic (microbial cell preparation) and omega-3 fatty acid in modulating quality of life and inflammatory markers in colorectal cancer patients: a randomized controlled trial." in: **Asia-Pacific journal of clinical oncology**, Vol. 14, Issue 3, pp. 179-191, (2018) (PubMed).

There are more publications referencing this product on: Product page

Images

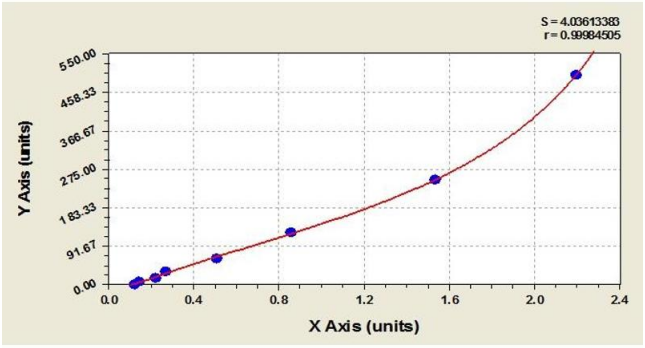
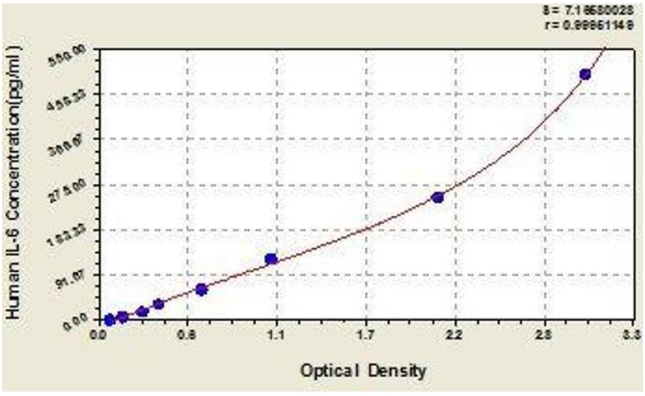


Image 1.



ELISA

Image 2. Typical standard curve

Validation report #028773 for ELISA (ELISA)

Successfully validated (ELISA (ELISA))

by Alamo Laboratories Inc

Report Number: 028773

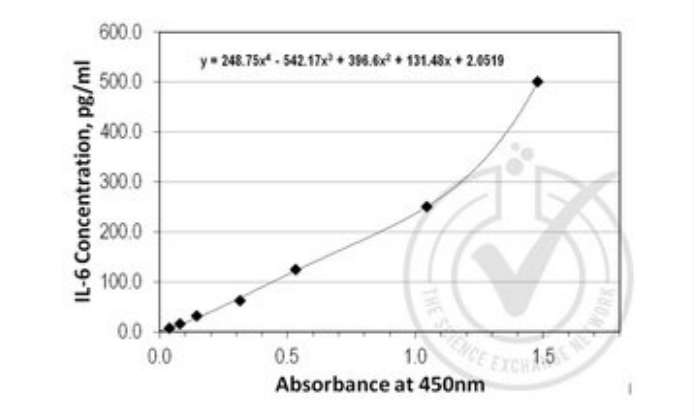
Date: Oct 02 2013

Lot Number:	Q03097538
Method validated:	ELISA (ELISA)
Positive Control:	Human serum
Negative Control:	Mouse brain lysate
Notes:	Signal was detected in positive control samples but not in negative control samples.
Primary Antibody:	- Antigen: Interleukin 6 (IL-6) - Catalog number: ABIN365163 - Supplier: Cusabio - Supplier catalog number: csb-e04638h - Batch number: Q03097538
Controls:	• Positive control: human serum (specimen known to contain the target protein) was prepared by Alamo • Laboratories. • Negative control: protein extract from mouse brain (specimen known to not contain the target protein) • was prepared by Alamo Laboratories. • Standard curve: serial two-fold dilutions from 500 pg/ml [500, 250, 125, 62.5, 31.25, 15.6, 7.8, 0] were • generated from the standard provided in the kit using sample diluent buffer. • Spike control: standard diluted in PBS [62.5 and 0 pg/mL].
Protocol:	• To each well, 100 µL of standard or sample were added. Plate was incubated for 2 h at 37°C. The • liquid from each well was aspirated but wells were not washed. • 100 µL of Biotin-antibody (1x) was added to each well and the plate was incubated for 1 h at 37°C. • The liquid from each well was aspirated and wells were washed three times with 200 µL of Wash Buffer • (1X) each time x 2 min. • 100 µL of HRP-avidin (1x) was added to each well and the plate was incubated for 1 h at 37°C. • The liquid from each well was aspirated and wells were washed five times with 200 µL of Wash Buffer • (1X) each time x 2 min.

- 90 µL of TMB Substrate was added to each well and the plate was incubated for 25 min at 37°C.
- 50 µL of Stop Solution to each well and the contents were mixed by tapping gently. Absorbance of each
- well was measured at 450 nm and 540 nm within 5 min using a microplate reader.
- The readings at 540 nm were subtracted from those at 450 nm to correct for optical imperfections in the
- plate.
- The triplicate readings for each sample were averaged and the average zero standard optical density
- subtracted. The corrected average-value was tabulated as Average Absorbance. A standard curve was
- generated by plotting the mean OD value for each standard on the X-axis against the concentration on
- the Y-axis using Excel. Standard curve was generated by regression analysis with four parameter
- logistic.
- An equation (y = 248.75x4-542.17x3+396.6x2+131.48x+2.0519) was derived from the standard
- curve and used to calculate IL-6 concentrations based on their Average Absorbance values.

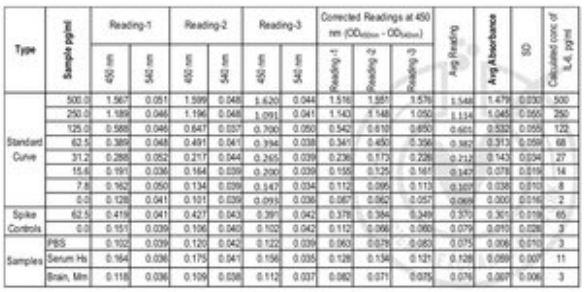
Experimental Notes:	None

Images for Validation report #028773



Validation image no. 1 for Interleukin 6 (IL6) ELISA Kit (ABIN365163)

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



Type	Sample pg/ml	Reading-1		Reading-2		Reading-3		Corrected Readings at 450 nm (OD_{450nm} - OD_{540nm})			Avg Reading	Avg Absorbance	SD	Calculated conc. of IL-6, pg/ml
		450 nm	540 nm	450 nm	540 nm	450 nm	540 nm	Reading -1	Reading -2	Reading -3				
Standard Curve	500.0	1.567	0.051	1.599	0.048	1.620	0.044	1.516	1.551	1.576	1.548	1.479	0.030	500
	250.0	1.189	0.046	1.196	0.048	1.091	0.041	1.143	1.148	1.050	1.114	1.045	0.055	250
	125.0	0.588	0.046	0.641	0.037	0.700	0.050	0.542	0.610	0.650	0.601	0.532	0.055	122
	62.5	0.389	0.048	0.491	0.041	0.394	0.038	0.341	0.450	0.356	0.382	0.313	0.059	68
	31.2	0.288	0.052	0.217	0.044	0.265	0.039	0.236	0.173	0.226	0.212	0.143	0.034	27
	15.6	0.191	0.036	0.164	0.039	0.200	0.039	0.155	0.125	0.161	0.147	0.078	0.019	14
	7.8	0.162	0.050	0.134	0.039	0.147	0.034	0.112	0.095	0.113	0.107	0.038	0.010	8
	0.0	0.128	0.041	0.101	0.039	0.091	0.036	0.087	0.062	0.055	0.068	0.000	0.016	2
Spike Controls	62.5	0.419	0.041	0.427	0.043	0.397	0.042	0.378	0.384	0.349	0.370	0.301	0.018	65
	0.0	0.151	0.039	0.106	0.040	0.102	0.042	0.112	0.066	0.060	0.079	0.010	0.026	3
Samples	PBS	0.102	0.039	0.120	0.042	0.122	0.039	0.063	0.078	0.083	0.075	0.006	0.010	3
	Serum Hs	0.164	0.036	0.175	0.041	0.156	0.035	0.128	0.134	0.121	0.128	0.089	0.007	11
	Brain, Mm	0.118	0.036	0.109	0.038	0.112	0.037	0.082	0.071	0.075	0.076	0.007	0.006	3

Validation image no. 2 for Interleukin 6 (IL6) ELISA Kit (ABIN365163)

Table 1: ELISA. IL-6 is present in the positive control sample (human serum) and absent from the negative control sample (mouse brain). Spike controls indicate no interference in absorbance readings from the protein lysate buffer used to prepare the protein extracts. Absorbance readings (OD 450 nm) are shown for standard curve, spike controls and unknown positive and negative control samples. The absorbance of all the samples including standards, spike controls and unknown samples were measured at 450 and 540 nm and the absorbance values at 540 nm were subtracted from those at 450 nm to account for optical imperfections in the ELISA plate. Value for Average Reading is derived from the average of three corrected-readings (OD 450nm). The Average Reading for 0 ng/ml Standard was subtracted from all Average Readings to yield Average Absorbance values for standards, spike controls and unknown samples. Standard deviation is included for all samples. Standard curve was generated by regression analysis with four-parameter logistic. An equation (y = 248.75x4 - 542.17x3 + 396.6x2 + 131.48x + 2.0519) was derived from the standard curve and used to calculate IL-6 concentrations shown in the Table.