WUHAN HUAMEI BIOTECH CO., LTD.
Postal Address: Wuhan Hi-tech Medical Devices Park, Building B11, #818 Gaoxin Road, Donghu Hi-Tech Development Area, Wuhan, Hubei Province 430206, P.R.China
Website: www.cusabio.com www.cusabio.cn
Email: cusabio@cusabio.com
Tel: +86-27-87582341
Fax: +86-27-87196150

REAGENT VALIDATION REPORT

SUMMARY

Antigen:	Interleukin 6 (IL-6)
Catalog number:	CSB-E04638h
Lot number:	Q03097538
Method validated:	Enzyme-linked immunosorbent assay
Date validated:	September 25, 2013
Laboratory:	Shakti Bioresearch
Validation number:	28769
Cell types:	U266 (positive)
	AsPC-1(negative)
Disclaimer*:	There is a possibility that results may vary between reagent lots.

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INDEPENDENT RESULTS

TABLE 1

Sample	Sample Description	OD 450nm	Conc (pg/mL)	MeanConc (pg/mL)	Std.Dev.	CV%	%Accuracy
AsPC	Cell extract diluted 20 fold	0.16	37.20	33.14	3.52	10.6	
		0.132	31.01				
		0.133	31.20				
AsPC_Sp	Spiked concentration of IL6 50 pg/mL	0.48	98.43	93.82	4.24	4.5	118.4
		0.449	92.94				111.8
		0.433	90.08				108.3
AsPC_Sup	Spent media diluted 10 fold	-0.023	BDL				
		-0.012	BDL				
		-0.023	BDL				
AsPC_Sup_Sp	Spiked concentration of IL6 50 pg/mL	0.274	60.51	47.04	11.94	25.4	121.0
		0.162	37.75				75.5
		0.186	42.87				85.7
U266	Cell extract diluted 20 fold	0.161	37.44	31.19	6.44	20.6	
		0.135	31.54				
		0.105	24.58				
U266_Sp	Spiked concentration of IL6 50 pg/mL	0.42	87.77	89.87	11.31	12.6	108.1
		0.376	79.76				98.2
		0.501	102.08				125.7
U266_Sup	Spent media diluted 10 fold	-0.024	BDL				
		-0.022	BDL				
		-0.02	BDL				
U266_Sup_Sp	Spiked concentration of IL6 50 pg/mL	0.215	48.82	44.36	4.74	10.7	97.6
		0.196	44.86				89.7
		0.17	39.40				78.8

Legend: ELISA. IL-6 is present in the positive control sample (U266 lysate 1.0 mg/mL) and in the negative control sample (AsPC-1 lysate 5.0 mg/mL) but at a 5-fold lower concentration (since IL-6 levels were ~30 pg/mL in 1.0 mg/mL of U266 and 5.0 mg/mL of AsPC-1). No IL-6 was identified in spent growth media collected from U266 and AsPC-1 cells. Spike controls indicate no interference in absorbance readings from the protein lysate buffer used to prepare the positive and negative control samples.Protein lysates were diluted 20-fold and spent media was diluted 10 fold for the ELISA assay. _Sp: lysates spiked with IL-6, _Sup: spent growth media, BDL: below detection limit.

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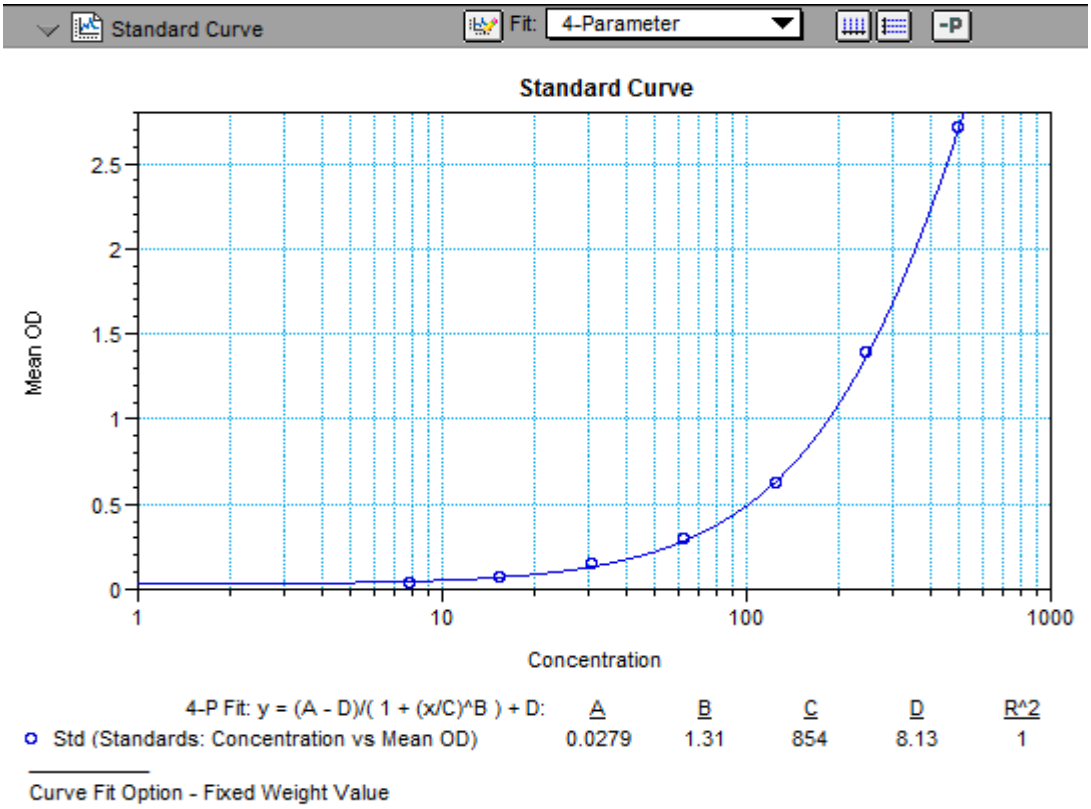
FIGURE 1



Sample	Concentration (pg/mL)	BackCalcConc (pg/mL)	Wells	OD 450nm	Mean OD	Std.Dev.	CV%	%Accuracy
St01	500	511.94	A1	2.772	2.715	0.204	7.5	102.4
		536.75	A2	2.885				107.4
		452.93	A3	2.489				90.6
St02	250	244.93	B1	1.351	1.385	0.226	16.3	98.0
		292.36	B2	1.627				116.9
		216.01	B3	1.178				86.4
St03	125	125.58	C1	0.638	0.622	0.087	14.1	100.5
		136.34	C2	0.701				109.1
		106.78	C3	0.528				85.4
St04	62.5	65.66	D1	0.301	0.29	0.017	5.9	105.0
		65.27	D2	0.299				104.4
		59.81	D3	0.27				95.7
St05	31.25	34.14	E1	0.146	0.145	0.003	2	109.3
		33.20	E2	0.142				106.2
		34.46	E3	0.147				110.3
St06	15.625	13.53	F1	0.063	0.067	0.005	7.6	86.6
		14.20	F2	0.066				90.9
		16.32	F3	0.073				104.4
St07	7.813	0.31	G1	0.028	0.039	0.01	26.2	4.0
		8.86	G2	0.048				113.4
		5.76	G3	0.04				73.7

Legend: Upper panel: graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples. Lower panel: table of absorbance readings (OD 450 nm) for standard curve. Value for Average Reading is derived from the average of three readings (OD 450nm). The Average Reading for 0 ng/ml Standard 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)^B) + D was generated from the standard curve and used to calculate IL-6 concentrations shown in Table 1.

FULL METHODS

ELISA

ELISA kitinformation

- Antigen: Interleukin-6 (IL-6)
- Catalog number: CSB-E04638h
- Batch number: Q03097538

Key experimental details

- The concentration of IL-6 in protein extracts from U266 cells and AsPC-1 cellswas measured using IL-6 ELISA kit.
- Standards provided in the kit were used to generate the standard curve.
- Detection: The optical density (OD value) of each well was read using a microplate reader set to 450nm and 570nm.

Controls

- Positive control: protein extract from U266 cells(specimen known to contain the target protein) was made at Shakti Bioresearch Labs and used at 1.0 mg/mL
- Negative control: protein extract from AsPC-1 cells(specimen known to not contain the target protein) was made at Shakti Bioresearch Labs and used at 5.0 mg/mL
- Standard curve: serial two-fold dilutions from 500 pg/ml [500, 250, 125, 62.5, 31.25, 15.625, 7.813, 0] were generated from the standard provided in the kit using sample diluent buffer.
- Spike control: standard diluted in protein lysate [50 pg/mL].

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Protocol

- All reagents in the ELISA kit were brought up to room temperature (RT) before use.
- 100 µl of each sample was added per well to the micro ELISA plate well. All samples and standards were assayed in triplicate.
- The plate was covered with sealer (provided in kit) and incubated for 120 mins at 37°C.
- Liquid was removed from each well by pipette.
- 100 µl of Biotinylated Detection Ab (diluted 1:100 in “Diluent for Biotinylated Detection Ab”) 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.
- 100 µl of HRP Conjugate (diluted 1:100 in “Diluent for HRP Conjugate”) was added to each well and the plate was sealed. The plate was tapped to ensure mixing and incubated for 60 min at 37°C.
- Wells were washed with 300 µl 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 for 20 min at 37°C in the dark.
- After 20 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 nm and 570nm.
- The triplicate readings for each sample were averaged and the average zero standard optical density subtracted. OD values at 570nm were subtracted from OD values at 450nm. 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)^B) + D was used to calculate IL-6 concentrations of the samples based on their average OD values.