Science EXCHANGE

Validation Report #028768

Validation Date: 09/27/13

Summary

Antigen	Interferon Regulatory Factor 4 (IRF4)
Catalog number	ABIN816582
Supplier	Cusabio
Supplier catalog number	csb-el011819mo
Lot number	Q17097537
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Shakti Bioresearch
Validation number	28768
Positive Control	Mouse spleen lysate
Negative Control	Mouse brain lysate
Notes	Signal was detected in positive control sample and not in negative control sample

Full Methods

Primary Antibody

- Antigen: Interferon Regulatory Factor 4 (IRF4)
- Catalog number: ABIN816582
- Supplier: Cusabio
- Supplier catalog number: csb-el011819mo
- Batch number: Q17097537

Controls

- Positive control: protein extract from mouse spleen (specimen known to contain the target protein) was from Abnova (Cat. No. L034W2, Lot No. TLD86195, 5 µg/mL)
- Negative control: protein extract from mouse brain (specimen known to not contain the target protein) was from Abnova (Cat. No. L035W2, Lot No. D9061, 11.41 µg/mL)
- Standard curve: serial two-fold dilutions from 1000 pg/ml [1000, 500, 250, 125, 62.5, 31.25, 15.625, 0] were generated from the standard provided in the kit using sample diluent buffer.
- Spike control: standard diluted in protein lysate [125 pg/mL].

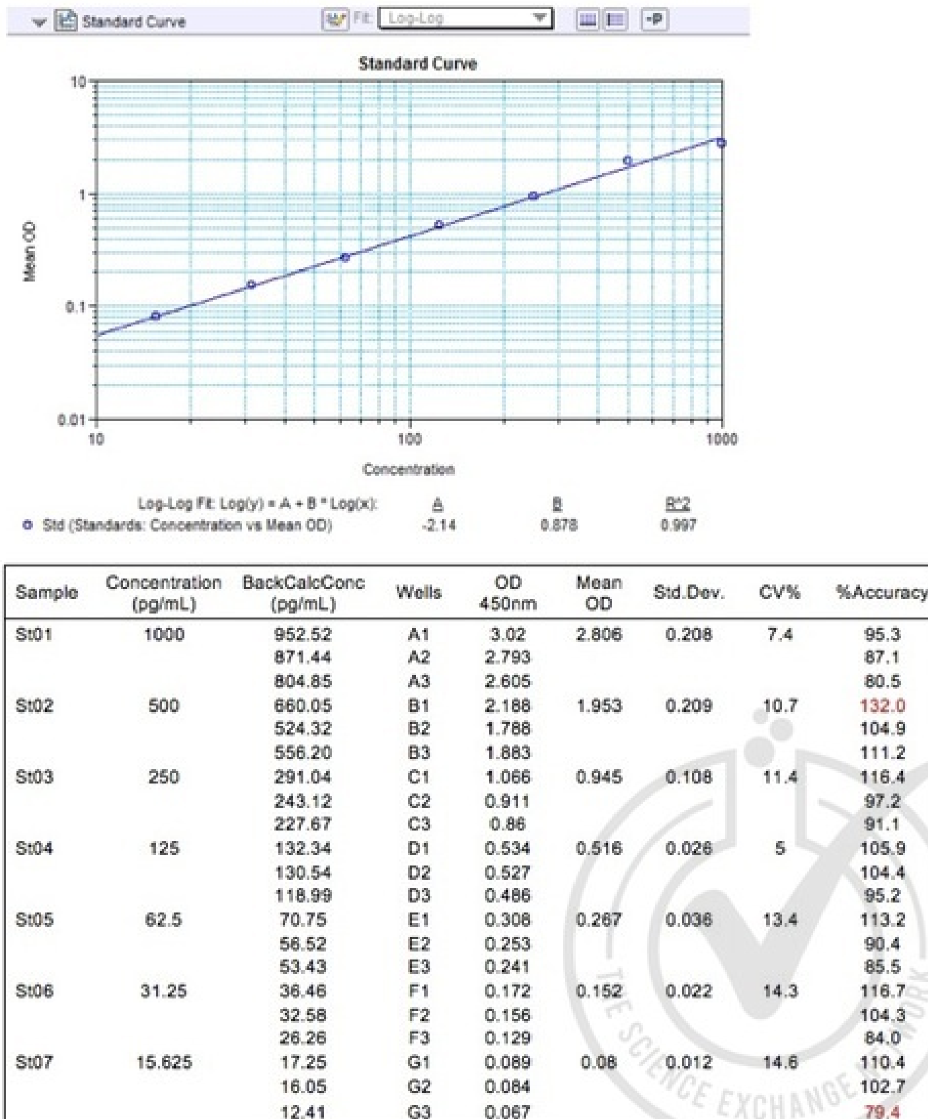
Protocol

- 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 min 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 min 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 min, 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 570 nm.
- The triplicate readings for each sample were averaged and the average zero standard optical density subtracted. OD values at 570 nm were subtracted from OD values at 450 nm. 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 software.
- The equation $\text{Log}(y) = A + B * \text{Log}(x)$ was used to calculate IRF4 concentrations of the samples based on their average OD values.

Experimental Notes

None

Figures



Sample	Concentration (pg/mL)	BackCalcConc (pg/mL)	Wells	OD 450nm	Mean OD	Std.Dev.	CV%	%Accuracy
St01	1000	952.52	A1	3.02	2.806	0.208	7.4	95.3
		871.44	A2	2.793				87.1
		804.85	A3	2.605				80.5
St02	500	660.05	B1	2.186	1.953	0.209	10.7	132.0
		524.32	B2	1.788				104.9
		556.20	B3	1.883				111.2
St03	250	291.04	C1	1.066	0.945	0.108	11.4	116.4
		243.12	C2	0.911				97.2
		227.67	C3	0.86				91.1
St04	125	132.34	D1	0.534	0.516	0.026	5	105.9
		130.54	D2	0.527				104.4
		118.99	D3	0.486				95.2
St05	62.5	70.75	E1	0.308	0.267	0.036	13.4	113.2
		56.52	E2	0.253				90.4
		53.43	E3	0.241				85.5
St06	31.25	36.46	F1	0.172	0.152	0.022	14.3	116.7
		32.58	F2	0.156				104.3
		26.26	F3	0.129				84.0
St07	15.625	17.25	G1	0.089	0.08	0.012	14.6	110.4
		16.05	G2	0.084				102.7
		12.41	G3	0.067				79.4

Figure 1: IRF4 ELISA. 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 450 nm). 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 Log(y) = A + B * Log(x) was generated from the standard curve and used to calculate IRF4 concentrations shown in Table 1.

Sample	Sample Description	OD 450nm	Conc (pg/mL)	MeanConc (pg/mL)	Std.Dev.	CV%	%Accuracy
Br_50	Brain extract diluted 50 fold	0.035	BDL				
		0.045	BDL				
		0.028	BDL				
Br_50_Sp	Spiked concentration of IRF4 125 pg/mL	0.504	124.05	124.89	6.03	4.8	99.2
		0.53	131.30				105.0
		0.487	119.32				95.5
Br_100	Brain extract diluted 100 fold	0.022	BDL				
		0.001	BDL				
		-0.005	BDL				
Br_100_Sp	Spiked concentration of IRF4 125 pg/mL	0.575	144.15	140.19	4.64	3.3	115.3
		0.544	135.09				108.1
		0.566	141.33				113.1
SP_50	Spleen extract diluted 50 fold	0.333	77.43	73.04	3.83	5.2	
		0.307	70.41				
		0.31	71.27				
SP_50_Sp	Spiked concentration of IRF4 125 pg/mL	0.981	264.58	223.21	37.00	16.6	133.6
		0.744	193.28				97.6
		0.807	211.78				106.9
SP_100	Spleen extract diluted 100 fold	0.276	62.39	57.97	5.68	9.8	
		0.266	59.97				
		0.233	51.56				
SP_100_Sp	Spiked concentration of IRF4 125 pg/mL	0.799	209.54	205.20	4.16	2	114.5
		0.771	201.26				110.0
		0.783	204.80				111.9

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Table 1: ELISA. IRF4 is present in the positive control sample (mouse spleen lysate) and absent from the negative control (mouse brain lysate) sample. Spike controls indicate no interference in absorbance readings from the protein lysate buffer used to prepare the positive and negative control samples. Br: Brain lysate, Sp: Spleen lysate, _50 or _100: fold dilution, _Sp: lysates spiked with IRF4, BDL: below detection limit.