Validation Report #029603

Validation Date: 02/10/14

Summary

Antigen	Human Transforming Growth Factor, beta 1 (TGFB1)
Catalog number	ABIN365402
Supplier	Cusabio
Supplier catalog number	CSB-E04725h
Lot number	T30095945
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Alamo Laboratories Inc
Validation number	29603
Positive Control	Human serum
Negative Control	Goat serum
Notes	Signal was detected in positive control sample and not in negative control sample.

Full Methods

Primary Antibody

- Antigen: Human Transforming Growth Factor, beta 1 (TGFB1)
- Catalog number: ABIN365402
- Supplier: Cusabio
- Supplier catalog number: CSB-E04725h
- Supplier lot number: T30095945

Controls

- Positive control: Serum from normal adult human (specimen known to contain the target protein).
- Negative control: Serum from normal goat (specimens known to not contain the target protein).
- Standard curve: Serial two-fold dilutions from 50 ng/ml [50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0] were generated from the standard provided in the kit using standard/sample diluent buffer.
- Spike control: Standard diluted in standard/PBS diluent buffer [3.12 and 0].

Protocol

- All reagents in the ELISA kit were brought up to room temperature (RT) before use.
- 80 µL of 1N HCl HEPES (provided in the kit) was added to 320 µL of human or goat sera and after 10 min incubation at RTP, 64 µL of 1.2N NaOH/0.5M HEPES (provided in the kit) was added to the mixture to complete the activation of sera. The activated sera samples were assayed immediately.
- 100 µL of standard or sample were added to wells in ELISA plate pre-coated with capture antibody. All samples and standards were assayed in triplicate.
- The plate was covered with sealer (provided in kit) and incubated for 2 hours at 37°C. Unbound material was aspirated but the wells were NOT Washed.
- 100 µL of Biotin-Antibody (diluted 1:100 in “Biotin-Antibody Diluent”) was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 hour at 37°C. Unbound Biotin-Antibody was removed from each well and plate was washed three times with 350 µL of wash buffer (provided in the kit). After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid.
- 100 µL of HRP-Avidin Conjugate (diluted 1:100 in “HRP-Avidin Diluent”) was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 hour at 37°C.
- Unbound HRP-Avidin was removed by washing five times with 350 µL of wash buffer (provided in the kit). After the last wash the plate was inverted and blotted against clean absorbent paper to remove any remaining liquid.
- 90 µL of TMB substrate was added to wells and the plate was covered with a new plate sealer. The plate was gently tapped to ensure mixing and incubated for 30 min at 37°C in the dark.
- After 30 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 microplate reader set to 450 nm.
- The triplicate readings for each sample were averaged and the average zero standard optical density subtracted to yield ‘corrected absorbance at 450 nm’. 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 = 35.792x4 - 29.65x3 + 19.134x2 + 34.232x) was derived from the standard curve and used to calculate TGF-beta1 concentrations in samples based on their Average Absorbance values.

Experimental Notes

None

Figures

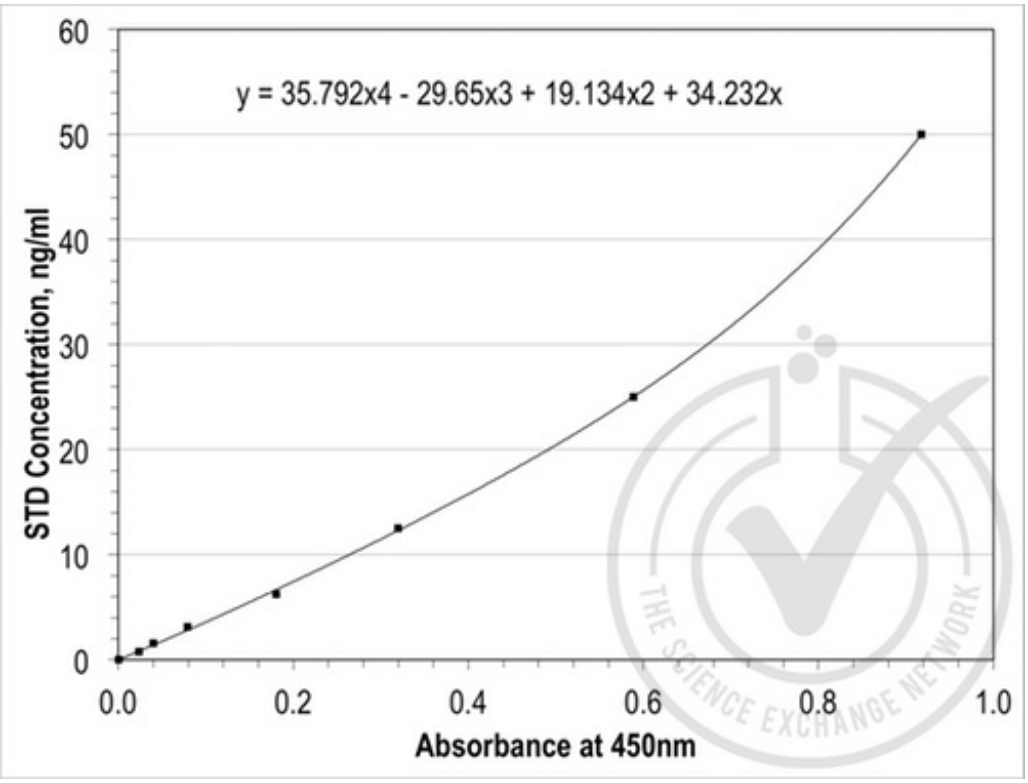

Figure 1: Graph of corrected OD 450 nm plotted for standard curve samples.

Type	Sample, ng/ml	Readings at 450 nm			Avg Reading	Corrected OD_{450nm}	SD	Calculated conc ng/ml
		1	2	3				
Standards	50	0.984	0.991	0.985	0.987	0.918	0.003	50.00
	25	0.689	0.629	0.655	0.658	0.589	0.025	25.03
	12.5	0.385	0.384	0.397	0.389	0.320	0.006	12.30
	6.25	0.236	0.256	0.257	0.250	0.181	0.010	6.67
	3.12	0.147	0.149	0.149	0.148	0.079	0.001	2.82
	1.56	0.113	0.112	0.102	0.109	0.040	0.005	1.40
	0.78	0.091	0.095	0.091	0.092	0.023	0.002	0.81
	0	0.074	0.067	0.067	0.069	0.000	0.003	0.01
Spike Controls	3.12	0.149	0.147	0.164	0.157	0.088	0.008	3.14
	0.00	0.074	0.074	0.075	0.074	0.005	0.000	0.18
Test Samples	Serum, Human, Activated	0.218	0.233	0.200	0.217	0.148	0.013	5.41
	Serum, Goat, Activated	0.069	0.062	0.068	0.066	-0.003	0.003	-0.09

Conc of TGF-β1 in Activated Human Serum (+ ve Control) : 5.41 ng/ml.
Conc of TGF-β1 in Activated Goat Serum (-ve Control) : - 0.09 ng/ml

Table 1: ELISA. TGF-beta1 is present in human serum and undetectable in goat serum. Spike controls indicate no interference in absorbance readings from the diluent used to prepare standards and sera samples. Absorbance readings (OD 450 nm) are shown for standard curve, spike controls and unknown samples. Value for Avg Reading is derived from the average reading of three samples. Avg Reading for “0” amount of Standard was subtracted from all Avg Readings to yield “Corrected OD 450 nm" 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 = 35.792x4 - 29.65x3 + 19.134x2 + 34.232x) was derived from the standard curve and used

to calculate TGF-beta1 concentrations shown in Table 1.