Validation Report #029832

Validation Date: 02/28/15

Summary

Antigen	Ki67P
Catalog number	ABIN415150
Supplier	Cloud Clone Corp.
Supplier catalog number	SEC047Hu
Lot number	L141016326
Method validated	Enzyme-linked immunosorbent assay
Laboratory	Alamo Laboratories
Validation number	029832
Positive Control	MCF7 lysate
Negative Control	Mouse brain lysate
Notes	Target protein was detected in the positive control sample and not in the negative control sample as expected.

Full Methods

ELISA kit

- Antigen: Ki67P
- Catalog number: ABIN415150
- Supplier: Cloud Clone Corp.
- Supplier catalog number: SEC047Hu
- Lot number: L141016326

Controls

- Positive control: MCF7 cell lysate prepared by lysis of cells by freeze-thaw cycles in phosphate buffered saline.
- Negative control: Mouse brain lysate prepared by lysis of tissue by freeze-thaw cycles in phosphate buffered saline

Protocol

All reagents in the ELISA kit were brought up to room temperature (RT) before use.
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 Detection Reagent-A Working Solution was added to each well. Plate was covered with sealer (provided in kit) and incubated for 1 hour at 37°C. Unbound material was removed from each well and plate was washed three times with 350 µL of 1x Wash Solution (provided in the kit). After the last wash the plate was inverted and blotted against clean absorbent paper to remove any remaining liquid.
100 µL of Detection Reagent-B Working Solution was added to each well. Plate was covered with sealer (provided in kit) and incubated for 30 minutes at 37°C.
Unbound material was removed by washing five times with 350 µL of 1x Wash Solution (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 Substrate Solution 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 25 minutes at 37°C in the dark.
After 25 minutes, 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 three-parameter logistic.
An equation (y = -4.6988x^3 + 14.578x^2 + 17.63x + 0.1488) was derived from the standard curve and used to calculate Ki67P concentrations in samples based on their Average Absorbance values.

Experimental Notes

No experimental challenges noted.

Figures

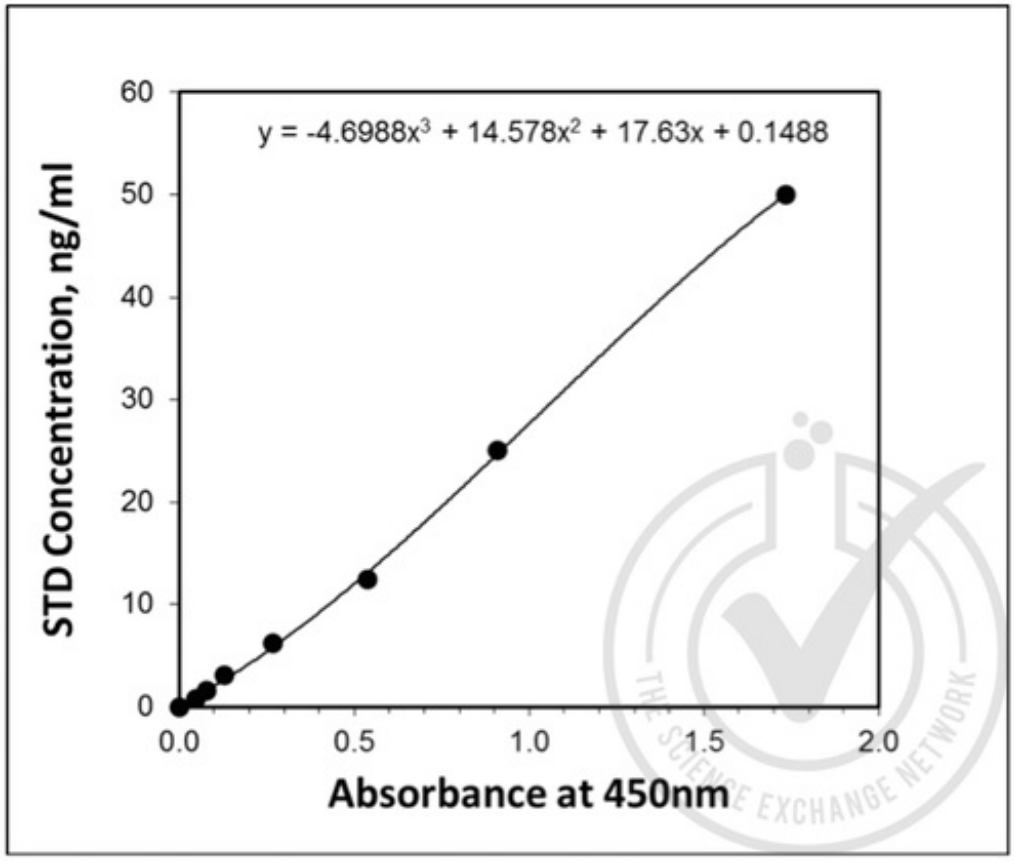

Figure 1: Graph of 'Corrected' OD450 nm plotted for standard curve samples. Standard curve was generated by regression analysis with three-parameter logistic. An equation (y = -4.6988x^3 + 14.578x^2 + 17.63x + 0.1488) was derived from the standard curve and used to calculate KI67P concentrations shown in Figure 2.

Type	Sample, ng/ml	Readings at 450 nm			Avg Reading	Corrected OD_{450nm}	SD	Calculated conc ng/ml
		1	2	3				
Standards	50	1.851	1.868	1.872	1.864	1.733	0.009	50.02
	25	1.067	1.035	1.023	1.042	0.911	0.019	24.74
	12.5	0.648	0.705	0.658	0.670	0.539	0.025	13.16
	6.25	0.397	0.406	0.393	0.399	0.268	0.005	5.82
	3.12	0.257	0.265	0.261	0.261	0.130	0.003	2.68
	1.56	0.202	0.222	0.202	0.209	0.078	0.009	1.60
	0.78	0.184	0.181	0.172	0.179	0.048	0.005	1.03
	0	0.131	0.134	0.129	**0.131**	0.000	0.002	0.15
Spike Controls	0.00	0.128	0.116	0.138	0.127	-0.004	0.009	0.08
	6.25	0.454	0.438	0.463	0.452	0.321	0.010	7.15
Test Samples	MCF7 Extract-PBS	0.652	0.736	0.781	0.723	0.592	0.053	14.72
	MCF7 Extract-PBS 1:4 Diluted	0.292	0.231	0.259	0.261	0.130	0.025	2.67
	Mm Brain Extract-PBS	0.141	0.128	0.130	0.133	0.002	0.006	0.18

Conc of Ki67P in MCF7 (+ve Control) 1:4 diluted: 2.67 x 4 = 11 ng/ml
Conc of Ki67P in MCF7 (+ve Control) undiluted: 15 ng/ml
Conc of Ki67P in Mouse Brain Extract: (-ve Control): 0.18 ng/ml (Below detection range of the kit)

Figure 2: Table of absorbance values for standard curve, spike control and 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 OD450 nm values” for Standards, spike controls and unknown samples. Standard deviation is included for all samples.