# Validation Report #029581

Validation Date: 01/26/14

## Summary

| | |
|---|---|
| Antigen | Human Chromosome 19 Open Reading Frame 80 (C19ORF80) |
| Catalog number | ABIN1136417 |
| Supplier | EIAab |
| Supplier catalog number | E11644h |
| Lot number | 3L306L |
| Method validated | Enzyme-linked immunosorbent assay |
| Laboratory | Shakti Bioresearch |
| Validation number | 29581 |
| Positive Control | Human serum |
| Negative Control | Mouse serum |
| Notes | Matrix interference indicates that serum must be diluted >10 fold for accurate measurement. Kit returned minor signal for negative control sample. |

# Full Methods

**Primary Antibody**

- Antigen: Human Chromosome 19 Open Reading Frame 80 (C19ORF80)
- Catalog number: E11644h
- Supplier: EIAAB Science Co.
- Lot number: 3L306L

**Controls**

- Positive control: normal human serum
- Negative control: mouse serum
- Standard curve: serial two-fold dilutions from 5000 pg/ml (5000, 2500, 1250, 625, 312.5, 156.25, 78.125, 0) were generated from the standard provided in the kit using sample diluent buffer.
- Spike control: standard diluted in human or mouse serum (500 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 mins at 37°C.
- Liquid was removed from each well by pipette.
- Detection Reagent A was diluted 100 fold in Assay Diluent A. 100 µl of diluted detection reagent A 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.
- Detection Reagent B was diluted 100 fold in Assay Diluent B. 100 µl of diluted detection reagent B 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 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 at room temperature in the dark.
- After about 10 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.
- The triplicate readings for each sample were averaged and the average zero standard optical density subtracted. 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.

**Experimental Notes**

Percent recovery of the spiked samples shows that there is matrix interference. Dilution of >10 fold is required for accurate measurement of the analyte in human serum samples.

## Figures

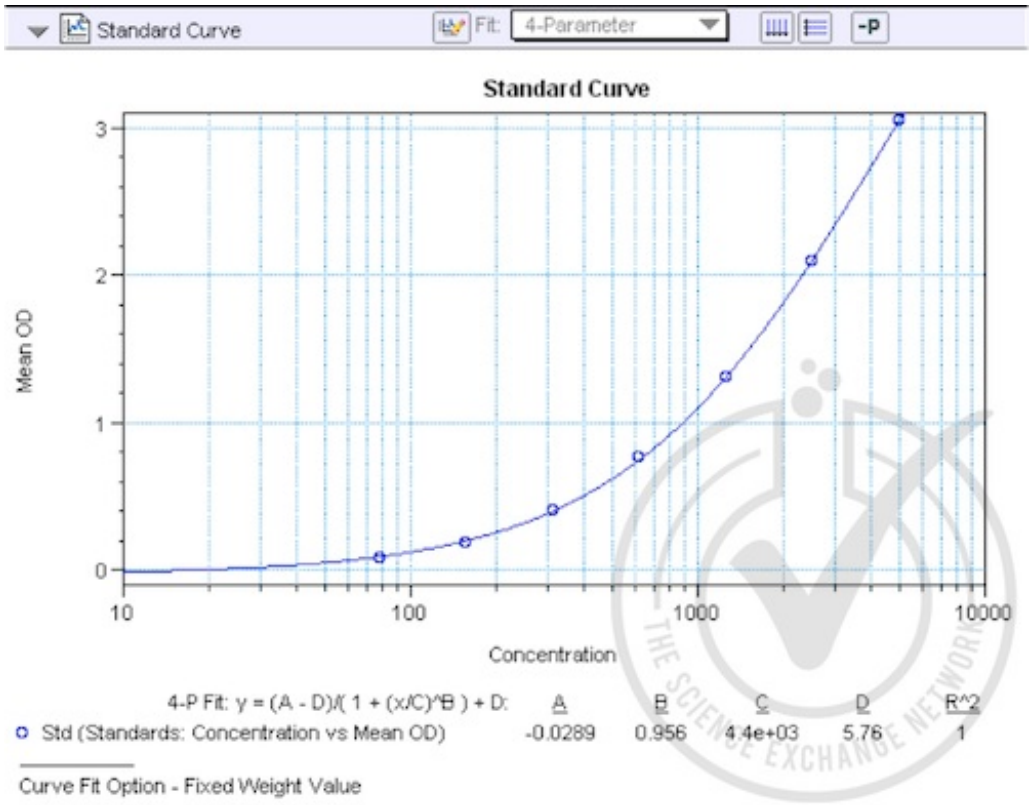


Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

| Sample Description | OD 450nm | Conc (pg/mL) | Mean Conc (pg/mL) | Std. Dev. | CV% | Dilution Factor | Conc (pg/mL) in neat serum | % Recovery |
|---|---|---|---|---|---|---|---|---|
| Human serum | 0.744 | 622.01 | 644.52 | 27.26 | 4.2 | | 644.52 | |
| | 0.759 | 636.73 | | | | | | |
| | 0.798 | 674.82 | | | | | | |
| 500 pg/mL of TD26 spiked into human serum | 0.652 | 534.74 | 628.86 | 152.32 | 24.2 | | | 54.9 |
| | 0.666 | 547.25 | | | | | | |
| | 0.924 | 804.60 | | | | | | |
| Human serum diluted 10 fold | 0.084 | 73.02 | 128.78 | 67.90 | 52.7 | 10 | 1287.75 | |
| | 0.135 | 108.91 | | | | | | |
| | 0.263 | 204.39 | | | | | | |
| 500 pg/mL of TD26 spiked into diluted human serum | 0.541 | 434.37 | 401.33 | 28.70 | 7.2 | | | 63.8 |
| | 0.487 | 387.10 | | | | | | |
| | 0.482 | 382.54 | | | | | | |
| Mouse serum | -0.008 | 12.16 | 11.17 | 1.35 | 12.1 | | 11.17 | |
| | -0.009 | 11.73 | | | | | | |
| | -0.012 | 9.64 | | | | | | |
| Mouse serum diluted 10 fold | -0.021 | 4.64 | 5.17 | 0.57 | 11 | 10 | 51.72 | |
| | -0.020 | 5.11 | | | | | | |
| | -0.019 | 5.77 | | | | | | |
| 500 pg/mL of TD26 spiked into diluted mouse serum | 0.550 | 442.33 | 355.25 | 101.26 | 28.5 | | | 70.3 |
| | 0.478 | 379.28 | | | | | | |
| | 0.314 | 244.14 | | | | | | |

Table 1: ELISA. C19ORF80 could be detected in human serum (positive control). Spike controls indicate that there is interference from the human serum matrix and a dilution of >10 fold is required. Mouse serum was used as negative control, there were residual levels of C19ORF80.

| Sample | Concentration (pg/mL) | BackCalcConc (pg/mL) | Wells | OD 450nm | Mean OD | Std.Dev. | CV% | %Accuracy |
|---|---|---|---|---|---|---|---|---|
| St01 | 5000 | 4979.52 | A1 | 3.038 | 3.047 | 0.009 | 0.3 | 99.6 |
| | | 5041.28 | A2 | 3.055 | | | | 100.8 |
| | | 5013.93 | A3 | 3.048 | | | | 100.3 |
| St02 | 2500 | 2293.61 | B1 | 1.993 | 2.094 | 0.132 | 6.3 | 91.7 |
| | | 2784.55 | B2 | 2.243 | | | | 111.4 |
| | | 2390.16 | B3 | 2.045 | | | | 95.6 |
| St03 | 1250 | 1318.03 | C1 | 1.361 | 1.31 | 0.068 | 5.2 | 105.4 |
| | | 1284.59 | C2 | 1.335 | | | | 102.8 |
| | | 1156.21 | C3 | 1.233 | | | | 92.5 |
| St04 | 625 | 684.81 | D1 | 0.808 | 0.76 | 0.041 | 5.4 | 109.6 |
| | | 613.97 | D2 | 0.736 | | | | 98.2 |
| | | 615.52 | D3 | 0.737 | | | | 98.5 |
| St05 | 312.5 | 353.49 | E1 | 0.448 | 0.402 | 0.04 | 10 | 113.1 |
| | | 300.25 | E2 | 0.384 | | | | 96.1 |
| | | 291.75 | E3 | 0.373 | | | | 93.4 |
| St06 | 156.25 | 135.41 | F1 | 0.171 | 0.186 | 0.019 | 10.3 | 86.7 |
| | | 162.34 | F2 | 0.208 | | | | 103.9 |
| | | 141.20 | F3 | 0.179 | | | | 90.4 |
| St07 | 78.125 | 73.51 | G1 | 0.085 | 0.08 | 0.005 | 6.8 | 94.1 |
| | | 71.92 | G2 | 0.082 | | | | 92.1 |
| | | 66.35 | G3 | 0.074 | | | | 84.9 |
| St08 | 0 | 12.72 | H1 | -0.007 | -0.017 | 0.008 | 48.1 | NA |
| | | 3.87 | H2 | -0.022 | | | | NA |
| | | 4.93 | H3 | -0.020 | | | | NA |

Table 2: 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 BLANK (0 pg/ml) was subtracted from all Average Readings to yield Average Absorbance values for Standards. Standard deviation is included for all samples. An equation (see Figure 1) was generated from the standard curve and used to calculate C19ORF80 concentrations shown in Table 1.