# Validation Report #029582

Validation Date: 01/24/14

## Summary


| | |
|---|---|
| Antigen | human Interleuken 8 (IL-8) |
| Catalog number | ABIN414887 |
| Supplier | USCNK |
| Supplier catalog number | SEA080Hu |
| Lot number | L131225502 |
| Method validated | Enzyme-linked immunosorbent assay |
| Laboratory | Shakti Bioresearch |
| Validation number | 29582 |
| Positive Control | Human serum |
| Negative Control | Mouse serum |
| Notes | The kit detected signal in positive control serum and did not detect signal in negative control serum. |

# Full Methods

**Primary Antibody**

- Antigen: Interleukin-8 (IL-8)
- Catalog number: ABIN414887
- Supplier: USCNK
- Supplier catalog number: SEA080Hu
- Lot number: L131225502

**Controls**

- Positive control: normal human serum
- Negative control: mouse serum
- Standard curve: serial two-fold dilutions from 1000 pg/ml [1000, 500, 250, 125, 62.5, 31.25, 15.6, 0] were generated from the standard provided in the kit using standard diluent buffer.
- Spike control: standard diluted in human or mouse serum [400 pg/mL].
- Lyophilized control provided by the kit was dissolved in 150 uL od standard diluent buffer (66.78 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 30 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-8 concentrations of the samples based on their average OD values.

**Experimental Notes**

Standards at lower level showed higher variability. Sensitivity of the assay needs to be improved as the human serum needs to be diluted >10 fold to avoid matrix interference.

**Isotype Control Antibody**

**Secondary Antibody**

**Additional Information**

## Figures

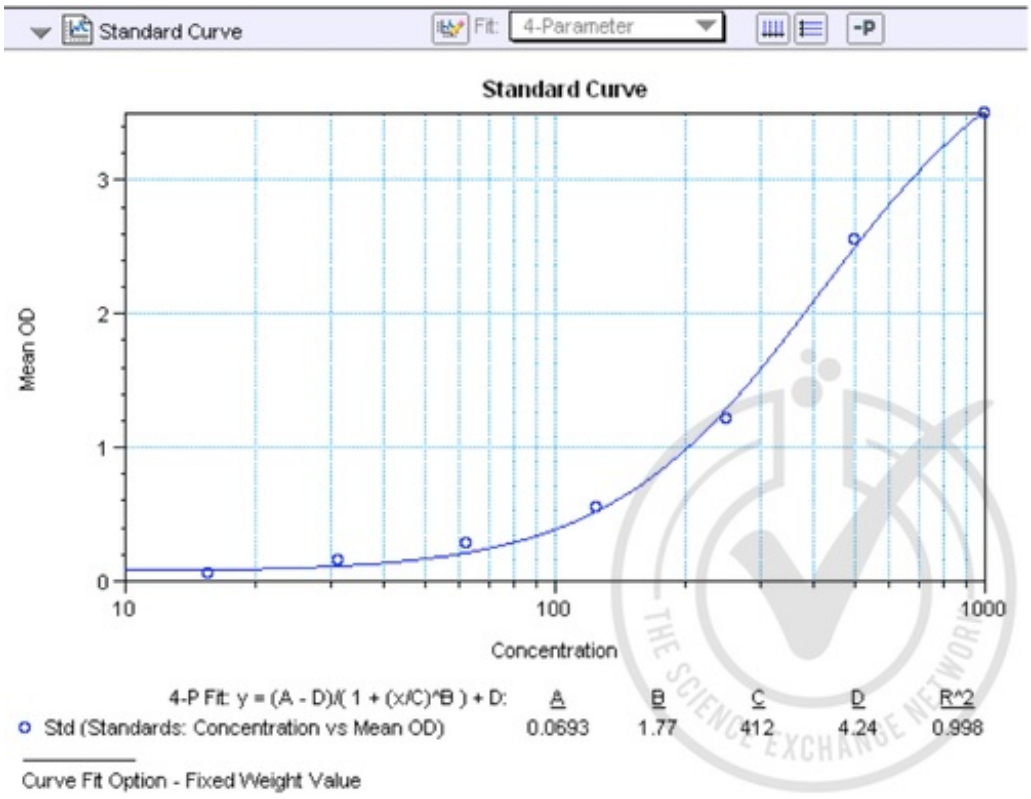


Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

| 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 diluted 2 fold | 0.195 | 57.63 | 53.39 | 7.13 | 13.4 | 2 | 106.78 | |
| | 0.194 | 57.39 | | | | | | |
| | 0.152 | 45.16 | | | | | | |
| 400 pg/mL of IL8 spiked into diluted human serum | 1.637 | 309.30 | 311.25 | 2.79 | 0.9 | | | 68.6 |
| | 1.666 | 314.45 | | | | | | |
| | 1.641 | 310.00 | | | | | | |
| Human serum diluted 10 fold | 0.024 | BDL | BDL | | | 10 | BDL | |
| | 0.018 | BDL | | | | | | |
| | 0.025 | BDL | | | | | | |
| 400 pg/mL of IL8 spiked into diluted human serum | 1.304 | 252.35 | 244.66 | 6.87 | 2.8 | | | 61.2 |
| | 1.223 | 239.12 | | | | | | |
| | 1.244 | 242.50 | | | | | | |
| Mouse serum diluted 2 fold | 0.005 | BDL | BDL | | | 10 | BDL | |
| | -0.004 | BDL | | | | | | |
| | 0.011 | BDL | | | | | | |
| Mouse serum diluted 10 fold | 0.007 | BDL | BDL | | | 50 | BDL | |
| | 0.008 | BDL | | | | | | |
| | 0.002 | BDL | | | | | | |
| 100 µL of Control sample tested | 0.409 | 104.61 | 104.61 | | | | 1046.09 | 1566.5 |
| 30 µL of Control sample tested | 0.147 | 43.81 | 43.81 | | | | 1460.33 | 2186.8 |
| 15 µL of Control sample tested | 0.070 | 1.82 | 1.82 | | | | 121.07 | 181.3 |

Table 1: ELISA. IL-8 could be detected in human serum (positive control) after 2 fold dilution. 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 no detectable levels of IL-8. BDL: below detection limit. Control sample concentration is expected to be 66.78 pg/mL.

| Sample | Concentration (pg/mL) | BackCalcConc (pg/mL) | OD 450nm | Mean OD | Std.Dev. | CV% | %Accuracy |
|---|---|---|---|---|---|---|---|
| St01 | 1000 | 1005.69 | 3.523 | 3.5 | 0.031 | 0.9 | 100.6 |
| | | 994.46 | 3.511 | | | | 99.4 |
| | | 951.86 | 3.464 | | | | 95.2 |
| St02 | 500 | 597.75 | 2.815 | 2.56 | 0.222 | 8.7 | 119.6 |
| | | 473.58 | 2.409 | | | | 94.7 |
| | | 485.87 | 2.455 | | | | 97.2 |
| St03 | 250 | 251.15 | 1.297 | 1.215 | 0.071 | 5.8 | 100.5 |
| | | 231.09 | 1.173 | | | | 92.4 |
| | | 231.46 | 1.176 | | | | 92.6 |
| St04 | 125 | 132.35 | 0.564 | 0.548 | 0.039 | 7.1 | 105.9 |
| | | 134.63 | 0.577 | | | | 107.7 |
| | | 121.86 | 0.504 | | | | 97.5 |
| St05 | 62.5 | 89.18 | 0.331 | 0.279 | 0.046 | 16.6 | 142.7 |
| | | 74.97 | 0.265 | | | | 119.9 |
| | | 69.45 | 0.241 | | | | 111.1 |
| St06 | 31.25 | 57.55 | 0.194 | 0.149 | 0.042 | 28 | 184.2 |
| | | 40.79 | 0.138 | | | | 130.5 |
| | | 31.43 | 0.113 | | | | 100.6 |
| St07 | 15.625 | 6.44 | 0.072 | 0.059 | 0.013 | 21.6 | 41.2 |
| | | Range? | 0.047 | | | | |
| | | Range? | 0.058 | | | | |
| St08 | 0 | Range? | 0.006 | 0.003 | 0.003 | 86.5 | |
| | | Range? | 0.002 | | | | |
| | | Range? | 0.001 | | | | |

Table 2: Generation of the 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 y = (A-D)/(1 + (x/C)^B) + D was generated from the standard curve and used to calculate IL-8 concentrations shown in the Figure 1 and Table 1.