# Validation Report #029635

Validation Date: 03/19/14

## Summary

| | |
|---|---|
| Antigen | C-Telopeptide of Type I Collagen |
| Catalog number | ABIN367349 |
| Supplier | Cusabio |
| Supplier catalog number | csb-e10363h |
| Lot number | 2014-2AX-2014-08AX |
| Method validated | Enzyme-linked immunosorbent assay |
| Laboratory | Affina Biotechnologies, Inc |
| Validation number | 029635 |
| Positive Control | Human serum |
| Negative Control | Chicken plasma |
| Notes | Signal was detected in positive control sample and not in negative control sample. |

# Full Methods

**Primary Antibody**

- Antibody: C-Telopeptide of Type I Collagen (ICTP)
- Catalog number: ABIN367349
- Supplier: Cusabio
- Supplier catalog number: csb-e10363h
- Lot number: 2014-2AX-2014-08AX

**Controls**

- Positive control: Human individual post-menopausal female serum (Biochemed, 750-NS-FI-POM) (positive)
- Negative control: Chicken plasma (Sigma-Aldrich, p3266) – reconstituted at 1 mg/mL
- Standard curve: 0, 25, 75, 175, 400, 800 ng/mL ICTP provided in the ELISA kit
- Spike control: 800 ng/mL standard premixed with chicken plasma in a 1:1 ratio

**Protocol**

- 50 µL of standard and samples were added 48-well strip plates provided in the kit with 50 µL of HRP conjugate. All samples and standards were assayed in duplicate.
- The microplate was covered and incubated at 37°C for 1 h.
- Plate contents were discarded.
- Content of the wells was discarded and wells were washed 3 times with 200 µL of distilled water with a 1 min soak for each wash.
- 50 µL of Substrate A and Substrate B each was added to each well. The plate was covered and incubated at 37°C for 15 min.
- 50 µL of the Stop Solution was added per well.
- The entire sample was transferred into a 96-well plate (Nunc, Maxisorp)
- The optical density (OD value) of each well was read immediately using a microplate reader set to 450 nm.
- The duplicate readings for each sample were averaged and the average zero standard optical density subtracted. The corrected average-value was tabulated as Average Absorbance. 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 CurveExpert 1.4. A linear equation was used for the best fit through the points on the graph.
- The CurveExpert Analyze feature was used to calculate human ICTP concentrations of the samples based on their Average Absorbance values.

**Experimental Notes**

- Transfers of final samples for OD reading were necessitated by inability to read a 48-well plate in the standard plate carriage. This caused high variance between duplicates.

## Figures

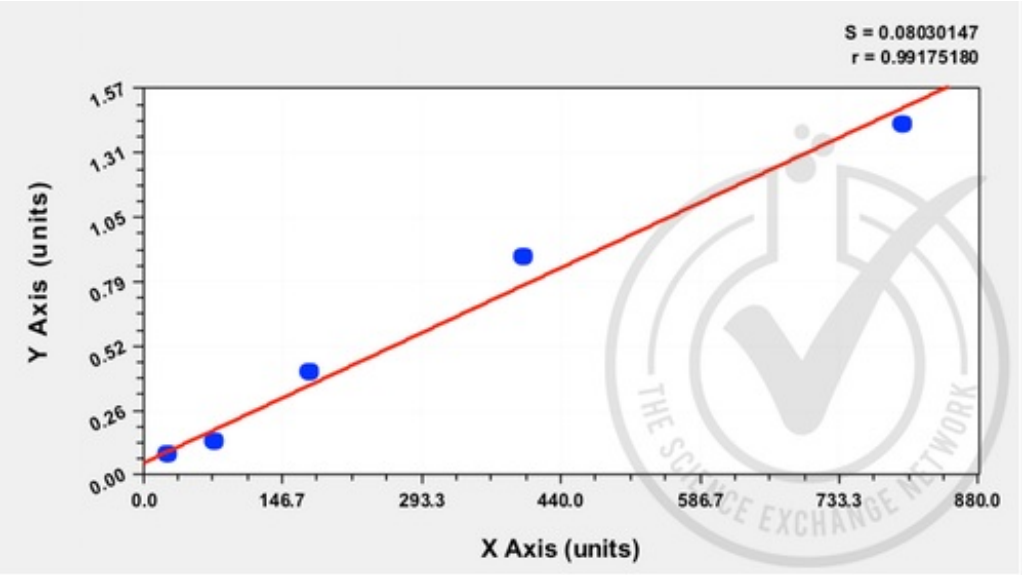


Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples. Equation: OD 450 = 5.00 + 1.804 * concentration

| Type | Sample ng/ml | Reading-1 | Reading-2 | Avg Reading | Avg Absorbance | SD | Calculated Conc |
|---|---|---|---|---|---|---|---|
| Standard Curve | 800 | 1.710 | 1.224 | 1.467 | 1.428 | 0.028 | 763.8 |
| | 400 | 1.014 | 0.839 | 0.927 | 0.888 | 0.028 | 464.6 |
| | 175 | 0.504 | 0.415 | 0.460 | 0.421 | 0.028 | 205.8 |
| | 75 | 0.149 | 0.199 | 0.174 | 0.135 | 0.028 | 47.3 |
| | 25 | 0.139 | 0.116 | 0.128 | 0.089 | 0.028 | 21.9 |
| | blank | 0.040 | 0.038 | 0.039 | | | |
| Spike Control | 400 | 1.157 | 1.059 | 1.108 | 1.069 | 0.070 | 565.0 |
| Positive Control | Human serum (1/2 diluted) | 0.623 | 0.584 | 0.604 | 0.5645 | 0.028 | 285.9 |
| Negative control | Chicken Plasma | 0.040 | 0.039 | 0.040 | 0.001 | 0.001 | -27.0 |

Table 1: Table of absorbance readings (OD 450 nm) for standard curve, spike controls and unknown control samples. Value for Average Reading is derived from the average of two readings (OD 450 nm). The Average Reading for blank sample (no conjugate added) was subtracted from all Average Readings to yield Average Absorbance values for Standards, spike controls and control samples. Standard deviation is included for all samples. The concentration of samples was calculated using the Analyze feature of the CurveExpert 1.4 software for a linear equation fit.