# Validation Report #029833

## Summary

| | |
|---|---|
| Antigen | Trypsin (TRY) |
| Catalog number | ABIN415936 |
| Supplier | Cloud Clone Corp. |
| Supplier catalog number | SEA250Po |
| Lot number | L141028604 |
| Method validated | Enzyme-linked immunosorbent assay |
| Laboratory | Alamo Laboratories |
| Validation number | 029833 |
| Positive Control | Trypsin from porcine pancreas |
| Negative Control | Chicken serum (non-reactive species) |
| Notes | Target protein was detected in the positive control sample and not in the negative control sample as expected. |

**INDEPENDENTLY VALIDATED**

**REPRODUCIBILITY INITIATIVE**

NO.: 029833   DATE: 03/02/15

# Full Methods

## ELISA kit
- Antigen: Trypsin (TRY)
- Catalog number: ABIN415936
- Supplier: Cloud Clone Corp.
- Supplier catalog number: SEA250Po
- Lot number: L141028604

## Controls
- Positive control: Trypsin from porcine pancreas (Sigma-Aldrich. Catalog # T6567) dissolved in STANDARD diluent (provided in the kit) by rocking the mixture for 30 minutes at room temperature.
- Negative control: Chicken serum (Jackson ImmunoResearch Laboratories Inc. Catalog #: 003-000-120, Lot #: 112503)

## 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.

An equation ($y = 67.557x - 1.4478$) was derived from the standard curve and used to calculate trypsin concentrations in samples based on their Average Absorbance values.

## Experimental Notes
The positive control was trypsin from porcine pancreas (Sigma-Aldrich Catalog # T6567). As per instruction from the manufacturer, the trypsin powder should be dissolved in 1 mM HCl. However, the acid solublization process renders the sample almost undetectable by the kit. Therefore trypsin powder was dissolved in STANDARD diluent (provided in the kit) by rocking the mixture for 30 minutes at room temperature.
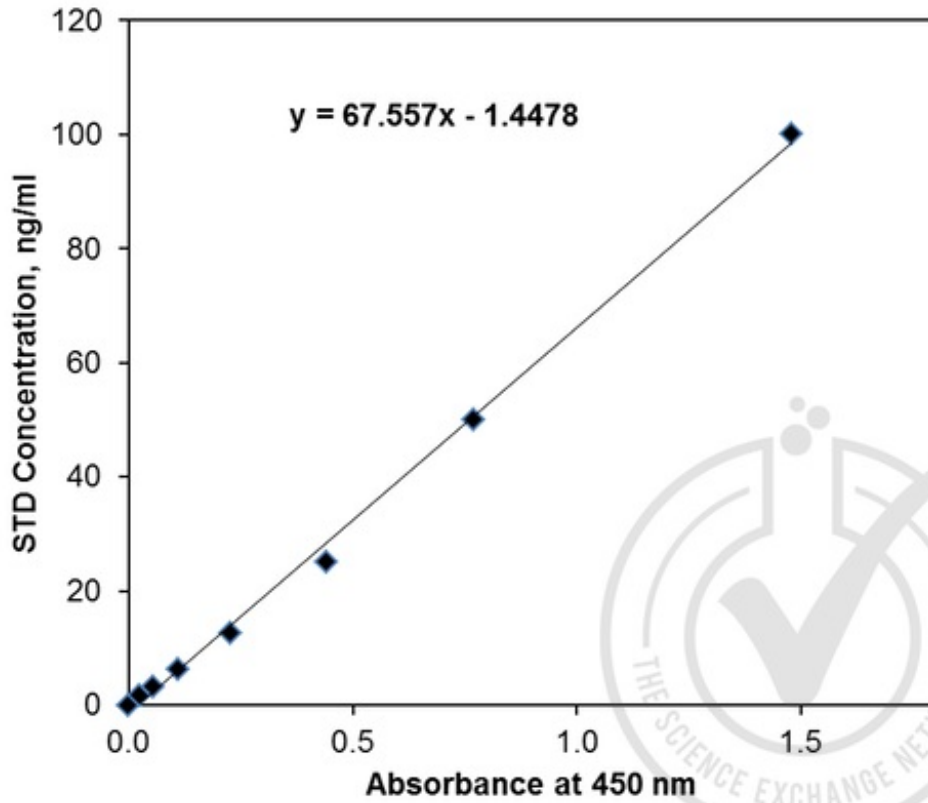
# Figures



Figure 1: Graph of 'Corrected' OD450 nm plotted for standard curve samples. Standard curve was generated by regression analysis. An equation (y = 67.557x - 1.4478) was derived from the standard curve and used to calculate Trypsin concentrations shown in Figure 2.

| Type | Sample, ng/ml | Readings at 450 nm | | | Avg Reading | Corrected OD450nm | SD | Calculated conc ng/ml |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | | |
| Standards | 100 | 1.554 | 1.540 | 1.564 | 1.553 | 1.480 | 0.010 | 98.51 |
| | 50 | 0.863 | 0.788 | 0.880 | 0.844 | 0.771 | 0.040 | 50.62 |
| | 25 | 0.516 | 0.526 | 0.503 | 0.515 | 0.442 | 0.009 | 28.41 |
| | 12.5 | 0.319 | 0.294 | 0.289 | 0.301 | 0.228 | 0.013 | 13.93 |
| | 6.25 | 0.189 | 0.190 | 0.170 | 0.183 | 0.110 | 0.009 | 5.98 |
| | 3.12 | 0.133 | 0.124 | 0.126 | 0.128 | 0.055 | 0.004 | 2.25 |
| | 1.56 | 0.092 | 0.090 | 0.108 | 0.097 | 0.024 | 0.008 | 0.15 |
| | 0 | 0.074 | 0.076 | 0.070 | 0.073 | 0.000 | 0.002 | -1.43 |
| Spike Controls | 0.00 | 0.079 | 0.074 | 0.070 | 0.074 | 0.001 | 0.004 | -1.36 |
| | 12.50 | 0.270 | 0.262 | 0.272 | 0.268 | 0.195 | 0.004 | 11.73 |
| Test Samples | Trypsin, 40 ng/ml, Porcine Sigma T6567 | 0.621 | 0.669 | 0.621 | 0.637 | 0.564 | 0.023 | 36.65 |
| | Trypsin, 20 ng/ml, Porcine Sigma T6567 | 0.345 | 0.364 | 0.347 | 0.352 | 0.279 | 0.009 | 17.40 |
| | Chicken Serum Undiluted | 0.107 | 0.083 | 0.094 | 0.095 | 0.022 | 0.010 | 0.02 |
| | Chicken Serum 1:1 Diluted | 0.097 | 0.063 | 0.111 | 0.090 | 0.017 | 0.020 | -0.28 |

Conc of 40 ng/ml Trypsin (+ve Control):   37 ng/ml
Conc of 20 ng/ml Trypsin (+ve Control):  17 ng/ml
Conc of Trypsin in chicken Serum (-ve Control):  0.02 ng/ml *Outside detection range of kit*
Conc of Trypsin in chicken Serum (-ve Control):  -0.28 ng/ml *Outside detection range of 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.