APPLICATION NOTE

# AUTOMATION OF THE ENZO APP ΔC31 ELISA KIT

## Introduction

Unlike the β-amyloid and tau fibril formation pathways leading to amyloid plaques and tangles, APP ΔC31 reveals an alternative, unique pro-apoptotic mechanism leading to Alzheimer's disease. APP ΔC31 is the stable amyloid precursor protein fragment created from a caspase cleavage event of the APP695 molecule at Asp664 leaving a smaller 31-residue intracellular fragment. Both the APP ΔC31 and 31-residue fragments are pro-apoptotic and are present in 4-fold greater levels in Alzheimer's disease patients. Given that the smaller 31-residue fragment has a short half-life and is difficult to measure, the use of this APP ΔC31 ELISA provides for the first time a sensitive research tool to measure the levels of the APP caspase cleavage from tissue, biological fluids, and cells.

The APP ΔC31 ELISA kit is a complete, colorimetric, immunometric immunoassay kit for the quantitative determination of human APP ΔC31 in cell lysate, serum, plasma, and cerebral spinal fluid samples with results in just 2 hours.

## Materials

- Crocodile miniWorkstation (Berthold Technologies)
- MikroWin module, quantitative and qualitative data reduction package (Titertek-Berthold)
- APP ΔC31 ELISA kit (#ADI-900-227, Enzo)
- ddH$_2$0, pipette and tips, sample tubs, Vortex mixer

## Methods

All reagents were brought up to room temperature for 30 minutes prior to use. According to manufacturer instructions wash buffer and 8 standards were prepared.

We changed the first steps in the assay procedure to have it more convenient for automation purposes: At the beginning 50µl of samples and standards in duplicate were transferred to the assay plate. For standard 0 (S0) 50µl of assay buffer was pipetted.

Automation with the Crocodile miniWorkstation was performed as shown on table 1 starting in dispensing antibody.

Adjust aspiration depth in your assay setup as to avoid cross-contamination through direct contact between aspiration needles and well surfaces.

The plate was read at 450 nm absorbance. After blanking the reader against the average blank OD, a standard curve was calculated by using MikroWin and fitted with four parameter algorithms.

## Results

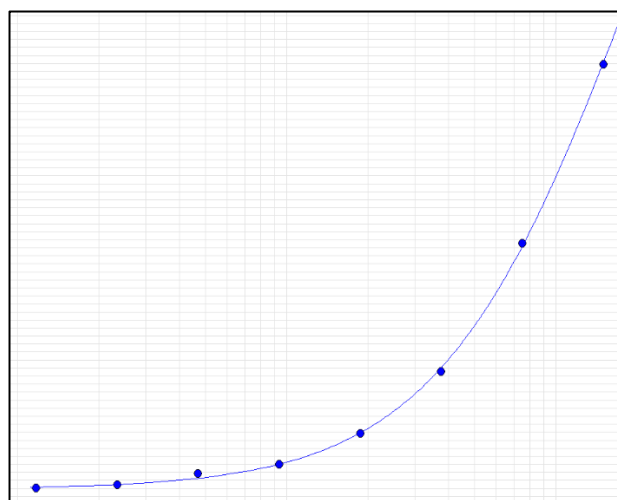| Standard | Concentration (pM) | OD average (Minus Blank OD) |
|----------|-------------------|------------------------------|
| Blank | - | 0 |
| S0 | 0 | -0.002 |
| S1 | 1500 | 2.135 |
| S2 | 750 | 1.242 |
| S3 | 375 | 0.602 |
| S4 | 187.5 | 0.293 |
| S5 | 93.75 | 0.139 |
| S6 | 46.88 | 0.091 |
| S7 | 23.44 | 0.036 |
| S8 | 11.72 | 0.020 |



*Figure 1. Standard curve fitted with four parameter algorithm. Y-axis linear, X-axis logarithmic.*

## Conclusion

The standard curve showed excellent fitting, so Crocodile provides a convenient and easy-to-use method for the automation of the Enzo APP ΔC31 ELISA kit. The assay procedure is extremely simple and involves only the addition of standards and controls while the instrument is processing all necessary dispense, wash, incubation and reading steps.

Changing the first steps in the assay procedure (pipetting antibody and standards/samples) doesn't affect the assay results at all.

## Acknowledgement

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Table 1: Assay program with Crocodile Control Software

| # | Step Name | Description |
|---|-----------|-------------|
| 1 | **Prime Antibody** | **Dispensing**<br>Volume 1000 µl Inlet 1 Label "Antibody"<br>Method: Priming Well Count: 1 |
| 2 | **Dispense Antibody** | **Dispensing**<br>Volume 50ul Inlet 1 Label "Antibody"<br>Method: Standard Well Count: 20 |
| 3 | **Incubate 1** | **Shaking**<br>for 01:00:00 at Incubator with 2mm Amplitude at 10 Hz |
| 4 | **Wash 1** | **Washing**<br>Method: Standard Wash Solution Inlet: 1 Wash buffer<br>Cycles: 3 Volume: 300 µl Delay: 2s Wait: 500ms<br>Dispenser Depth: 1300 (Plate Offset 50) Aspiration Depth 2725 (Plate Offset 20)<br>Sweep 5mm @1mm/s Well Count 96 |
| 5 | **Prime Conjugate** | **Dispensing**<br>Volume 1000 µl Inlet 2 Label "Conjugate"<br>Method: Priming Well Count: 1 |
| 6 | **Dispense Conjugate** | **Dispensing**<br>Volume 100ul Inlet 2 Label "Conjugate"<br>Method: Standard Well Count: 20 |
| 7 | **Incubate 2** | **Shaking**<br>for 00:30:00 at Incubator with 2mm Amplitude at 10 Hz |
| 8 | **Wash 2** | **Washing**<br>Method: Standard Wash Solution Inlet: 1 Wash buffer<br>Cycles: 3 Volume: 300 µl Delay: 2s Wait: 500ms<br>Dispenser Depth: 1300 (Plate Offset 50) Aspiration Depth 2725 (Plate Offset 20)<br>Sweep 5mm @1mm/s Well Count 96 |
| 9 | **Prime Substrate** | **Dispensing**<br>Volume 1000 µl Inlet 3 Label "Substrate"<br>Method: Priming Well Count: 1 |
| 10 | **Dispense Substrate** | **Dispensing**<br>Volume 100ul Inlet 3 Label "Substrate"<br>Method: Standard Well Count: 20 |
| 11 | **Incubate 3** | **Shaking**<br>for 00:30:00 at Incubator with 2mm Amplitude at 10 Hz |
| 12 | **Prime Stop** | **Dispensing**<br>Volume 1000 µl Inlet 4 Label "Stop"<br>Method: Priming Well Count: 1 |
| 13 | **Dispense Stop** | **Dispensing**<br>Volume 100ul Inlet 4 Label "Stop"<br>Method: Standard Well Count: 20 |
| 14 | **Measure** | **Reading**<br>Single Wavelength Filter 1: 450nm (Pos:2) Well Count: 96 |