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Berthold Detection Systems GmbH
Bleichstrasse 56–68
D-75173 Pforzheim/Germany

Phone: +49(0)7231/9206-0
Fax: +49(0)7231/9206-50
E-Mail: contact@titertek-berthold.com
Internet: www.titertek-berthold.com

Crocodile miniWorkstation
Crocodile Control Software/MikroWin

Automation of the DLD Diagnostika Serotonin ELISA using the Crocodile miniWorkstation

Introduction

Serotonin or 5-Hydroxytryptamine is a neurotransmitter and hormone, biochemically derived from Tryptophan. Serotonin is primarily found in the gastrointestinal tract, blood platelets, and the central nervous system of animals, including humans. Central-serotonergic neurons influence physiological functions such as sleep and the hormonal and cardio-vascular regulation. Increased serum levels can be found with malignant carcinoid, endogenous depression and schizophrenia.
The DLD Serotonin enzyme immunoassay (EIA) provides material for the quantitative measurement of derivated Serotonin (5-Hydroxytryptamine) in serum, plasma and urine. The derivation is performed during preparation of the samples by using acylation reagent. Serotonin is bound to the solid phase of the microtiter plate and both acylated and solid phase bound Serotonin compete to the antibody binding sites. The amount of antibody bound to the solid phase Serotonin is inversely proportional to the Serotonin concentration of the sample.

Materials

- Crocodile miniWorkstation (Titertek-Berthold)
- Serotonin-ELISA (Ref EA602/96, DLD Diagnostika GmbH)
- ddH_2O, pipette and tips, Vortex mixer

Methods

All reagents were brought up to room temperature for 30 minutes prior to use.
According to manufacturer instructions wash buffer and reagents were prepared and acylation of all samples, standards and controls was performed. Each 20µl of acylated samples, standards and controls was transferred to the assay plate.

Automation with the Crocodile miniWorkstation was performed as shown on table 1. Make sure to properly adjust aspiration depth in your assay setup as to avoid cross-contamination through direct contact between aspiration needles and well surfaces.

A standard curve was calculated by using MikroWin 2010 and fitted with four parameter algorithm.

Results:

Standard	Concentration (ng/ml)	OD average (450nm)
1	0	1,607
2	15	1,424
3	50	1,143
4	150	0,782
5	500	0,397
6	2500	0,171

Control	Target Value (ng/ml)	Target range (ng/ml)	Calculated concentrations (ng/ml)
1 (low)	99	64-134	106
2 (high)	347	226-468	407

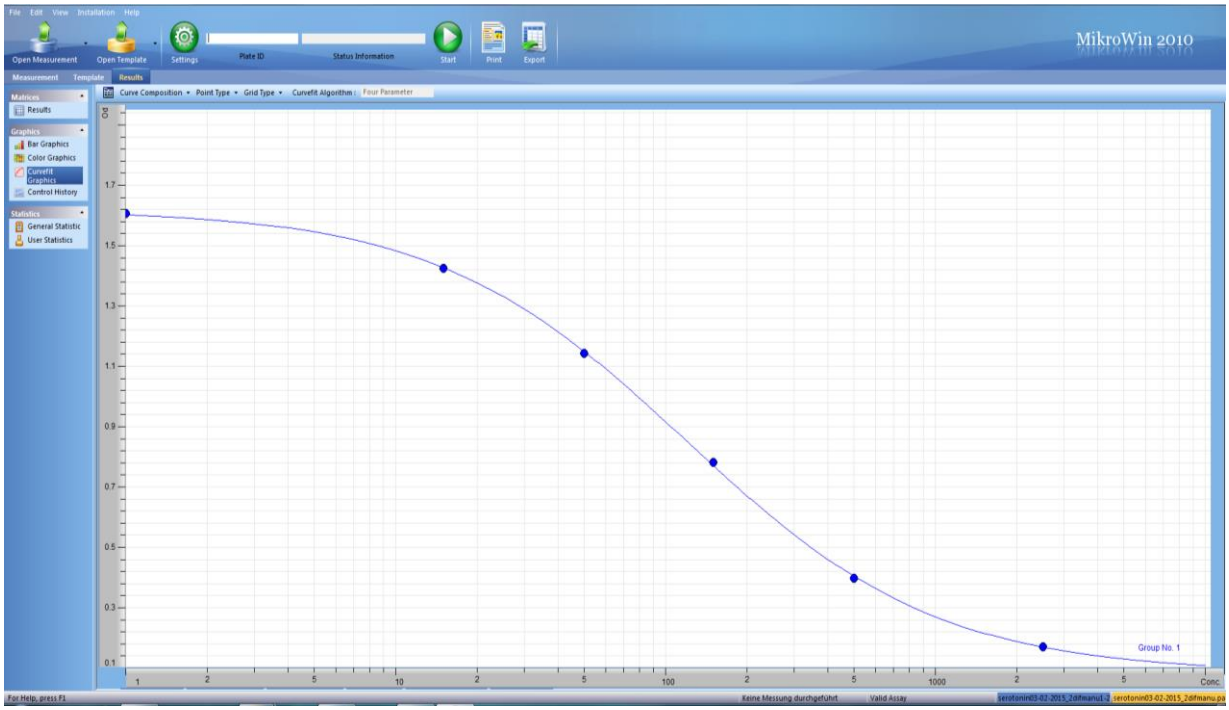

Figure 1. Standard curve fitted with four parameter algorithm. Y-axis linear, X-axis logarithmic.

Conclusion:

Using the Crocodile miniWorkstation for the assay procedure is extremely simple and involves only the addition of standards and controls. Crocodile provides a convenient and easy-to use method for the automation of the DLD Serotonin ELISA kit. The standard curve showed excellent fitting and low and high control met the target range.

Acknowledgement: Special thanks to DLD Diagnostika for their support.

#	Step Name	Description
1	**Prime Antiserum**	**Dispensing** Volume 850ul Inlet 1 Label "Antiserum AS " Method: Priming Well Count: 0
2	**Dispense Antiserum**	**Dispensing** Volume 100ul Inlet 1 Label "Antiserum AS " Method: Standard Well Count: 32
3	**Shake 1**	**Shaking** for 00:00:10 at Shaker Position with 1mm Amplitude at 5Hz
4	**Incubation 1**	**Incubation** Incubator On Temperature: 22.5°C Duration: 00:30:00
5	**Prime Wash**	**Washing** Method: Prime Dispenser Wash Solution Inlet: 1 Wash buffer WASH Cycles: 3 Volume: 800ul Delay: 1s Wait: 100ms Dispenser Depth: 1300 (Plate Offset: 0) Aspiration Depth: 1300 (Plate Offset: 0) Well Count: 32
6	**Wash 1**	**Washing** Method: Standard Wash Solution Inlet: 1 Wash buffer WASH Cycles: 4 Volume: 250ul Delay: 1s Wait: 200ms Dispenser Depth: 1300 (Plate Offset: 0) Aspiration Depth: 2890 (Plate Offset: 0) Sweep: 4mm @ 2mm/s Well Count: 32
7	**Aspirate only 1**	**Washing** Method: Aspirate Only Wash Solution Inlet: 1 Wash buffer WASH Cycles: 1 Volume: 100ul Delay: 1s Wait: 500ms Dispenser Depth: 1300 (Plate Offset: 0) Aspiration Depth: 2890 (Plate Offset: 0) Sweep: 4mm @ 2mm/s Well Count: 32
8	**Prime Conjugate**	**Dispensing** Volume 850ul Inlet 2 Label "Enzyme conjugate CONJ " Method: Priming Well Count: 0
9	**Dispense Conjugate**	**Dispensing** Volume 100ul Inlet 2 Label "Enzyme conjugate CONJ " Method: Standard Well Count: 32
10	**Shake 2**	**Shaking** for 00:00:10 at Shaker Position with 1mm Amplitude at 5Hz
11	**Incubation 2**	**Incubation** Incubator On Temperature: 22.5°C Duration: 00:15:00
12	**Wash 2**	**Washing** Method: Standard Wash Solution Inlet: 1 Wash buffer WASH Cycles: 4 Volume: 250ul Delay: 1s Wait: 200ms Dispenser Depth: 1300 (Plate Offset: 0) Aspiration Depth: 2890 (Plate Offset: 0) Sweep: 4mm @ 2mm/s Well Count: 32
13	**Aspirate only 2**	**Washing** Method: Aspirate Only Wash Solution Inlet: 1 Wash buffer WASH Cycles: 1 Volume: 100ul Delay: 1s Wait: 500ms Dispenser Depth: 1300 (Plate Offset: 0) Aspiration Depth: 2890 (Plate Offset: 0) Well Count: 32
14	**Prime Substrate**	**Dispensing** Volume 850ul Inlet 3 Label "Substrate SUB " Method: Priming Well Count: 0
15	**Dispense Substrate**	**Dispensing** Volume 100ul Inlet 3 Label "Substrate SUB " Method: Standard Well Count: 32
16	**Incubate with Shaking**	**Shaking** for 00:15:00 at Incubator with 1mm Amplitude at 5Hz
17	**Prime Stop**	**Dispensing** Volume 850ul Inlet 4 Label "Stop solution. STOP " Method: Priming Well Count: 0
18	**Dispense Stop**	**Dispensing** Volume 100ul Inlet 4 Label "Stop solution. STOP " Method: Standard Well Count: 32
19	**Shake 3**	**Shaking** for 00:00:10 at Shaker Position with 1mm Amplitude at 5Hz
	Measure	**Reading** Reference Measurement Filter 1: 450nm (Pos:2) Filter 2: 620 nm (Pos: 4) Well Count: 32

Table 1: Assay program with Crocodile Control Software