

## Crocodile miniWorkstation Crocodile Control Software

### Automation of the Invitron Intact Proinsulin ELISA using the Crocodile miniWorkstation

#### Introduction

Proinsulin is a precursor molecule for insulin and is synthesized by the pancreatic  $\beta$ -cells. Under normal circumstances, virtually all proinsulin is cleaved at residues 32-33 and 65-66 to produce insulin during the formation of secretory granules. Some unmodified proinsulin is released into the circulation, though it is believed to have little or no biological activity. Increased concentrations of circulating proinsulin may occur in insulin resistant syndromes such as type 2 diabetes and in patients with insulinoma.

The Invitron Intact Proinsulin Assay is a two-site enzyme-linked immunosorbent assay (ELISA), employing a specific solid phase antibody immobilized on microtiter wells and soluble antibody labeled with HRP enzyme.

#### Materials

##### 1. Crocodile miniWorkstation by Titertek-Berthold

The Crocodile miniWorkstation combines the functionality of 5 individual instruments in the footprint of the size of a standard stand-alone ELISA reader.



##### 2. Intact Proinsulin ELISA by Invitron

Invitron's Intact Proinsulin ELISA (2<sup>nd</sup> generation) is a kit for the quantitative measurement of intact proinsulin.

Format: 96 well plate

Specimen: Plasma or serum

Sample volume: 50  $\mu$ l

Working range: 0 - 100 pmol/l, Sensitivity: 0.3 pmol/l

Specificity: Proinsulin 100%, 32-33 split proinsulin 5.6%, des 31/32 proinsulin 1.4%, insulin 0%, C-peptide, 0%



##### 3. ddH<sub>2</sub>O, pipette and tips, Vortex mixer

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### Methods:

The table below illustrates the assay protocol for the Invitron Intact Proinsulin ELISA (2<sup>nd</sup> generation) when automated using the Crocodile miniWorkstation.

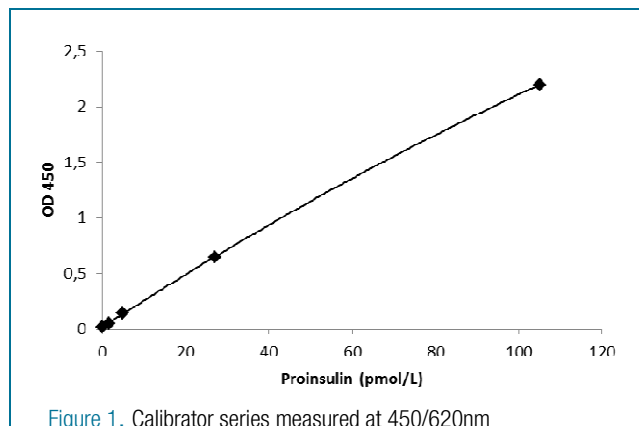
Step	User Action	Automated Action	Time
1	Prepare assay reagents and load them onto Crocodile.		10 min <sup>1</sup>
2	Pipette calibrators, controls and samples into the microplate and place the plate in the Crocodile.		20 min <sup>1</sup>
3	Start Crocodile control software, select the required assay protocol, enter assay meta data and press start.	Instrument wakes up, software prompts user for meta data and stores the information entered.	5 min <sup>1</sup>
4		Add sample buffer to each well	
5		Incubate	
6		Wash microplate (x5)	
7		Add antibody conjugate to each well	
8		Incubate	
9		Wash microplate (x5)	
10		Add substrate to each well	
11		Incubate	
12		Add stop solution	
13	View assay results.	Read microplate at 450/620nm, display assay results on screen and save data.	
14	Start Crocodile result viewer to carry out further functions such as calculating sample concentrations.	Open datafile and prepare for transfer to secondary application, e.g. excel or curve fit interpolation software.	
User hands-on time			35 min <sup>1</sup>

<sup>1</sup> Approximate time (exact time is user dependent)

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### Results:

Calibrator	Concentration (pmol/l)	OD (450-620)	Precision (duplicates CV %)
1	0	0.019	3.8
2	1.6	0.052	2.7
3	5.0	0.134	1.1
4	27	0.659	2.7
5	105	2.176	0.3



### Conclusion:

The Crocodile miniWorkstation provides a convenient and easy to use method for automation of the Invitron intact Proinsulin ELISA. During a parallel comparison, the precision and accuracy of results produced by the Crocodile were equivalent or better than an assay run manually by a trained technician<sup>2</sup>. Total assay time for the automated assay is approximately the same as when the assay is performed manually but operator hands-on time is reduced to just 35 minutes<sup>1</sup>.

<sup>1</sup> Approximate time (exact time is user dependent)

<sup>2</sup> Data available on request

Acknowledgement: Data provided by Invitron. Special thanks to Andrew Woodhead from Invitron for generous support.

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