



# ELEGANCE NEONATAL IRT ELISA KIT

**REF** 40 500480

**Σ** 480

**REF** 40 502400

**Σ** 2400



## WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions.

The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

The manufacturer disclaims any and all implied warranties of merchantability, fitness for use or implied utility for any other purposes. Any and all damages for failure of the diagnostic kit to perform according to its instructions are limited to the replacement value of the kit.

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## INTENDED USE

The *ELEGANCE* Neonatal IRT ELISA has been designed for the quantitative *in vitro* diagnostic measurement of immunoreactive trypsinogen (IRT) in the screening of human neonatal blood spots.

## PRINCIPLES OF THE ELEGANCE ELISA

The *ELEGANCE* ELISA is an enzyme-linked immunoassay. The IRT is eluted from the blood spot and is "sandwiched" between the antibody bound to the microwell and the biotinylated antibody reagent. The microwells are washed to remove any unbound material. Streptavidin-peroxidase (Amplification Reagent) is added and binds to the biotinylated antibody at many sites. After washing, the Substrate Solution reacts with any bound peroxidase to produce colour in direct proportion with the amount of sample antigen, which can be calculated from the calibrator curve.

## ELEGANCE REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 480 tests and 2400 tests (in parentheses).

The kit and all its components, unopened or opened, should be stored at 2-8°C until the listed expiry dates.

## Neonatal IRT:

### Coated Microwells

**1 x 96 wells REF # ITA96**

**5 x 96 wells REF # ITA5  
(25 x 96 wells REF # ITA25)**

Frame containing microwells coated with anti-IRT antibody. Ready to use.

### Neonatal IRT:

#### Antibody Reagent

**1 vial REF # ITB480**

**(1 vial REF # ITB25)**

50 (250) mL biotinylated anti-IRT antibody in a buffered solution containing bovine serum albumin and a blue dye. Contains sodium azide,

0.1% w/v. Ready to use.

### Neonatal IRT:

#### Amplification Reagent

**1 vial REF # ITP480**

**(1 vial REF # ITP25)**

50 (250) mL streptavidin-peroxidase (streptavidin from *S. avidinii*) in a buffered solution containing bovine serum albumin and a violet dye. Contains Bronidox L, 0.2% v/v and thiomersal, 0.02% w/v. Ready to use.

#### Wash Concentrate

**1 vial REF # EWC5**

**(2 vials REF # EWC25)**

250 (500) mL of a 15 x concentrated wash solution. Contains thiomersal, 0.09% w/v. To be diluted before use.

#### Stabilizing Stop Solution

**1 vial REF # ESCL5**

**(1 vial REF # ESCL25)**

30 (124) mL 2M HCl.

Ready to use.

## Substrate Solution TMB N

**1 vial REF # TMBB5**

**(1 vial REF # TMBB25)**

50 (250) mL 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide in a stabilising solution.

Ready to use.

## Neonatal IRT: Calibrators

Available as either:

**6 vials REF # EITS7**

**(2 x 6 vials REF # EITS7)**

0.5 mL each in buffer solution containing BSA and a yellow dye. Contains sodium azide, 0.2% w/v and thiomersal, 0.01% w/v. Lyophilized.

Or:

## Blood Spot Calibrators and Controls

**1 set REF # EIBS6**

**(4 sets REF # EIBS7)**

Dried human IRT blood spots with 6 calibrators (A-F) and 2 controls (1-2) on filter paper. Ready to use. (A further Top Calibrator [nominally 1000 µg/L] and Control are available on request).

## PRECAUTIONS AND WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

## Specimens and Calibrators

The source material of the calibrators have been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and have been found to be non-reactive for all. However it is recommended that all samples be handled as if capable of transmitting infectious disease.

## Preservatives

The kit contains sodium azide, thiomersal and Bronidox L as preservatives. As reagents contain potentially toxic preservatives, care should be taken in handling, to avoid ingestion or skin contact. Sodium azide may react with lead and copper plumbing to form potentially explosive azides.

## Substrate Solution and Stabilizing Stop Solution

Avoid any skin contact.

## SPECIMEN COLLECTION AND HANDLING

The *ELEGANCE* Neonatal IRT ELISA is intended for use with neonatal heel prick blood samples collected and dried on filter paper according to NCCLS LA4A Guidelines, where applicable. The filter paper should be Schleicher and Schuell Grade 903.

Blood from a neonatal heel prick is collected 3-5 days after birth.

A blood spot covering one circular sample area on the filter paper is obtained by one application of the filter paper onto a drop of blood flowing from the pricked heel of the baby.

The filter paper sample area should be fully covered and soaked through. After collection of the test samples, the filter papers are dried horizontally (2 hours or more). The dry specimens can be stored at 2-8°C. Only blood spots prepared in the above manner should be tested. Unused filter paper should be desiccated at room temperature (20-25°C).

## MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- \* Distilled or deionised water
- \* Disc punch (3mm)
- \* Forceps
- \* Precision pipettes
- \* Repeating pipette
- \* 1L measuring cylinder (lint-free)
- \* Absorbent tissue
- \* Timer
- \* Microtitre plate shaker
- \* Microtitre plate washer
- \* Microplate reader system.

## PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use. A calibrator curve should be run with each assay. Duplicates are recommended. Contamination of reagents will lead to poor performance. Prior to first wash, punched discs should be removed either by suction or gentle pin prick. All assay steps should be performed without interruption, but if the wells cannot be filled with Amplification Reagent or Substrate Solution immediately after washing, then the microwells may be left upside down on absorbent lint-free tissue for a maximum of 30 minutes.

Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed. The photometer and all pipettes used should be calibrated appropriately before use.

## Washing

The efficiency of the wash step is vital for good precision. Microwells are washed using an automatic plate washer. Avoid overflows from one well to another.

## Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

## ASSAY PROCEDURE

### Preparation of Reagents

#### Wash Solution

Dilute the wash concentrate 1 in 15 with deionised water. The wash solution can be stored at room temperature (20-25°C) for 12 weeks.

#### Lyophilized Calibrators\*

To reconstitute the lyophilized calibrators, add the volume of deionised water indicated on each vial label. Allow the vials to sit undisturbed until completely dissolved (at least 30 minutes) and then mix by gentle inversion. Exact concentrations determined lot-to-lot are stated on a separate label inside the kit. After reconstitution, the calibrators should be stored at -20°C for 8 weeks.

#### Blood Spot Calibrators and Controls\*

Exact concentrations determined lot-to-lot are stated on a label for each set of blood spots. To avoid condensation, do not open blood spots before temperature equilibration. Reseal unused blood spot strips in plastic bag and store at 2-8°C.

#### Protocol

1. Assemble the microwells in the frame according to the number of tests required. Bag and return unused wells to 2-8°C.
2. Place a single 3 mm punched disc (control or specimen) into appropriate wells. Avoid the edge of the blood spot when punching the sample.
3. Pipette 15 µL or a single 3mm punched disc of each calibrator (as provided) into the appropriate wells. Time taken to dispense any liquid calibrators should not exceed 20 minutes.
4. Pipette 100 µL of Neonatal IRT Antibody Reagent (blue) into all wells.
5. Cover microwells with lid and incubate for 10 minutes on a plate shaker at room temperature (20-25°C).
6. Incubate microwells overnight (16-24 hours) stationary at room temperature (20-25°C).
7. Next day incubate for 10 minutes on a plate shaker at room temperature (20-25°C).
8. After incubation, remove discs.
9. Wash the microwells. Aspirate the liquid and rinse each well 4 times with 250 µL of wash solution. After the final wash, invert the microwells and tap firmly on absorbent tissue to remove any remaining wash solution. Ensure that no air bubbles remain in the wells.
10. Pipette 100 µL of Neonatal IRT Amplification Reagent (violet) into each well.

11. Cover microwells with lid and incubate for 10 minutes on a plate shaker at room temperature (20-25°C).

12. After incubation, repeat wash step.

13. Pipette 100 µL of Substrate Solution into all wells. Timing of the incubation step is measured from the addition of Substrate Solution to the first well.

14. Cover microwells with lid and incubate for 10 minutes stationary at room temperature (20-25°C).

15. Pipette 50 µL of Stabilizing Stop Solution into all wells in the same timed sequence as for Substrate Solution addition.

16. Carry out an end-point reading at 450 nm and process data as described in the microplate reader user's manual. This reading step should be carried out within 30 minutes of stopping the reaction.

## CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction. Determine the OD for each well. Plot the calibrator curve using log-log graph paper with concentration of calibrators on the x-axis and OD on the y-axis. The curve may be drawn point-to-point or a curve-fitting routine, such as spline interpolation, may be used. Interpolate the sample values from OD measured from this calibrator curve. Record the value for each sample in µg/L whole blood IRT. The range of the *ELEGANCE* Neonatal IRT ELISA is from 0 to approx. 1000\* µg/L whole blood, but the maximum concentration that can be reported is limited by the linear performance characteristics of the photometer used. If the OD value of the highest calibrator is above the range of the photometer, then this calibrator must be omitted from the plot of the calibrator curve. Similarly any sample measuring above the range of the microplate reader should be simply noted as greater than the highest acceptable calibrator.

## MODEL CALCULATIONS

Endpoint Data	ID	Ave OD	IRT (µg/L W.B.)
	0	0.054	
	13.2	0.507	
	30.8	1.169	
	67.8	2.011	
	161	2.629	
	331	3.052	
	1041*	3.513	
	Sample 1	1.630	46.8
	Sample 2	2.315	103.8
	Sample 3	2.930	271.8

## CALIBRATION

The calibrators supplied in this kit are calibrated based on protein measurement and are expressed in µg/L whole blood. It is assumed that the volume of blood in a 3 mm blood spot is 3 µL with a haematocrit value of 55% (v/v). Conversion of standard units may be made using the following relationship:

$$\mu\text{g/L serum} = \mu\text{g/L whole blood} \times 2.2$$

## EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. Typically, a cut-off value of 50 µg/L whole blood is used for infants 3-5 days after birth. Samples measuring lower than the presumptive positive cut-off are considered normal. It has been found that trypsinogen concentrations in unaffected infants were not normally distributed but could be converted to a normal distribution by a logarithmic transformation.

## PERFORMANCE CHARACTERISTICS

### Intra-assay Precision

Sample	n	Mean ± 2SD (µg/L whole blood)	%CV
1	20	33.1 ± 1.8	2.8
2	20	58.0 ± 5.7	4.9
3	20	14.9 ± 2.4	7.9

### Inter-assay Precision

Sample	n *	Mean ± 2SD (µg/L whole blood)	%CV
1	66	28.0 ± 2.8	5.0
2	66	56.4 ± 6.2	5.5
3	66	15.8 ± 3.5	11.0

\* duplicate

### Specificity

Analyte	Concentration Assayed	Apparent IRT (µg/L W.B.)	Xreactivity
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#### Human

α <sub>2</sub> -Macroglobulin	50 mg/mL	<1	N.D
α-1-Antitrypsin	3 mg/mL	<1	N.D
α-Chymotrypsin A	780 ng/mL	<1	N.D

#### Bovine

Trypsinogen	500 ng/mL	<1	N.D
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### Accuracy

Recovery was calculated by assaying before and after addition of exogenous analyte.

Sample	Observed IRT (µg/L W.B.)	Expected IRT (µg/L W.B.)	% Recovery
1	95.3	94.7	100.6
2	154.0	153.0	100.7
3	259.7	226.0	114.9

### Dilution

A sample was diluted in zero calibrator, assayed and recovery calculated.

Sample	Observed IRT (µg/L W.B.)	Expected IRT (µg/L W.B.)	% Recovery
Neat	196.7		
1/2	98.0	98.4	99.6
1/4	49.7	49.2	101.0
1/8	24.5	24.6	99.6

### High-dose Hook Effect

Hook effects are characteristic of the assays and may occur when high value samples yield aberrant IRT results. No hook effect, was found for test samples with IRT values up to and including the test limit of 80000 µg/L whole blood.

### Sensitivity

The sensitivity of the assay is typically less than 1 µg/L whole blood (2.2 µg/L serum). The sensitivity is defined as that concentration of analyte which corresponds to the dose response variable (OD) that is two standard deviations from the mean dose response variable of 26 replicate determinations of the zero calibrator run in three different assays.

## ORDERING INFORMATION

The *ELEGANCE* NEONATAL IRT ELISA is manufactured by: Bioclone Australia Pty Limited, 71-73 Railway Parade, Marrickville, NSW 2204, AUSTRALIA. Telephone +61 (0) 2 9517 1966 Freecall 1800 251 138 Fax +61 (0) 2 9517 2990 Email sales@bioclone.com.au Web www.bioclone.com.au

## TECHNICAL SERVICE

Full technical service is available by calling Bioclone on +61 (0) 2 9517 1966 or Freecall 1800 251 138

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(\* if applicable)