DRG® Trypsin Neonate ELISA (EIA-1278)

USA: RUO

Revised 30 Sept. 2010 rm (Vers. 2.1)

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

The Neonate Trypsin ELISA kit is for measurement of human immunoreactive trypsin (IRT) from blood spot samples collected on Schleicher and Schuell's filter paper #903. This kit is intended for Research Use Only.

PRINCIPLE OF THE NEONATE TRYPSIN ELISA

The Neonate Trypsin ELISA kit employs an enzyme-linked immunosorbent assay (ELISA) technique to quantitate human Trypsin in a blood spot sample. In ELISA assays, two complementary antibody configurations are generated against different portions of the same antigen. In the ELISA, one antibody system is bound to the micro-plate well and the other antibody is labeled with an enzyme. When antigen is present, it simultaneously binds both antibodies in a "bridge" or "sandwich" fashion. This entire complex remains bound to the well. After washing out "unbound" enzyme, a specific substrate is added and converted to a colored end product and the reaction is rapidly terminated with stopping solution. The absorbance is read for each well at 450 nm and the results plotted as concentration of IRT in ng/mL versus absorbance on graph paper.

In the procedure, a disk is punched from a blood spot collected on Schleicher and Schuell's filter paper #903. This disk is placed into the antibody well along with an eluting buffer. After an overnight incubation, the eluting buffer and blood spot are aspirated out, the well is washed, and enzyme-labeled antibody is added. After a second incubation, the well is washed, and substrate is added to the well. The enzyme reaction is rapidly terminated with stopping solution and the absorbance is read. A standard curve is then constructed from which unknown concentrations of trypsin can be calculated.

The Rabbit Anti-IRT Antibody

Most significantly, the IRT ELISA utilizes the IRT antibody with its unique specificity characteristics with respect to the "molecular forms" of IRT. (23-27)

Since the original development, of the polyclonal antibody to IRT, we have reported variously on unique aspects of the antibody in its enhanced reactivity with a "pathological" form of IRT relative to the normal circulating form. This "pathological specificity" is independent of quantitative differences in absolute value of the standards used and is a function of the antibody. Results of extensive analysis of the antibody suggest that "pathologic IRT"-- with respect to its molecular species or interactions -- demonstrates greater "potency" with our antibody than does purified intact trypsin.

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REAGENTS INCLUDED IN THE KIT

The following reagents are included in the Single Assay Blood Spot Trypsin ELISA

A. **Trypsin-ELISA Enzyme Conjugate Concentrate (CONJ ENZ)** 0.5 mL
Horse-radish peroxidase conjugated to monoclonal anti-trypsin antibody in PBS, pH 7.4.

For use, pour the entire contents of the Enzyme Diluent vial into the vial of Enzyme Concentrate, cap vial, and invert gently several times to mix.
Stability of diluted reagent is 1 week (7 days) at 2-8°C.
Stability of sealed Enzyme Concentrate is as indicted on label.

NOTE: Care should be taken when removing the vial cap that no enzyme concentrate clinging to the cap is lost. If more than one vial of enzyme reagent is required, pool and mix all diluted enzyme prior to use.

B. **Enzyme Diluent (DILUENT ENZ)** 22.0 mL
Stability, when used with enzyme concentrate is 1 week at 2-8°C.

C. **Eluting Buffer (BUFFER ELUTION)** 40.0 mL
Stability of buffer is as per label at 2-8°C.

D. **Wash Buffer Concentrate (BUFFER WASH)** 50.0 mL
Phosphate buffer containing 0.5% Tween 20.

Dilute 10 times (to 500 mLs) with distilled water prior to use.
Stability of diluted wash buffer is per kit shelf life at 2-8°C.

E. **Color Substrate (SUB COLOR)** 22.0 mL
Ready-to-use 3,3',5,5'-tetramethylbenzidine (TMB).
Stability of TMB after opening is 1 week at 2-8°C.

F. **Stopping Solution (SOLN STOP)** 22.0 mL
H_2SO_4 in deionized water.
Stability is as per label at 2-8°C.

G. Polyclonal Anti-human Trypsin Coated **Microwell Plates (ANTI WELLS)** (96 wells) 2 each
Stability of unopened strips is as per kit expiration date at 2-8°C after opening sealed foil pouch.

H. **Standards (STD 1-7)** [7 levels], **Controls (CONTROL 1-3)** [3 levels] 1 card each
Whole blood spiked with human trypsin spotted on filter paper. Concentrations are as indicated on label.
Stability of unopened card is as indicated on label.
Stability of card after opening pouch is as per kit shelf life at less than -15°C.

NOTE: Actual calibration levels may change between lots, the label on the current lot of Standards should be consulted for calibration values to be used in calculations.

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WARNING: HUMAN BASED MATERIALS
Handle as if capable of transmitting infection. Source material from which this product was derived was found non-reactive for HIV1, HIV2 antibody and non-reactive for HBsAg and Hepatitis C when tested with licensed reagents at the donor level. No known test method can offer assurance that products derived from human blood will not be infectious. Refer to CPC/NIH Bio-Safety in Microbiological and Biochemical Laboratories publication (HHS Publication No. CPC 84-8395).

WARNING: SODIUM AZIDE
These reagents contain sodium azide which has a tendency to build up in lead or copper plumbing forming potentially explosive metal-azides. Always flush large quantities of water through the plumbing after the disposal of these reagents.

CALIBRATION AND STANDARDIZATION

The quantity of IRT in the sample (blood spot) is calculated from a standard curve prepared from a known amount of Trypsin calibrated by comparison with our well-established blood spot IRT RIA's. Currently, no international reference preparation exists for Trypsin.

EQUIPMENT AND REAGENTS REQUIRED

A. Plate Reader able to read absorbance at 450 nm.

B. Multi-channel and single micro-pipets calibrated to 100, 200, and 300 µL.
NOTE: 200 µL or 300 µL is acceptable for wash step.

C. Automated plate washer (optional).

COLLECTION AND HANDLING OF BLOOD SPECIMENS

Transfer enough blood to filter paper to completely fill at least two circles. It is absolutely essential that blood penetrates the filter paper to the other side. Blood should be spotted in the center of the circle and allowed to diffuse outward. Avoid tearing or disrupting the filter paper surface.
Allow specimen to air dry completely (overnight) and do not place near heat, in direct sunlight, or on absorbent surfaces. After drying overnight, specimens should be stored in an air-tight plastic envelope at less than –15°C until assay.

ASSAY STEPS

(Duplicates Recommended)

NOTE: To be used for a maximum of two consecutive plates. For larger assays, the timing of pipetting should be lagged to insure uniform plate processing.

For each calibrator, control and unknown, in duplicate:

1. Punch one ⅛" (3 mm) blood soaked filter paper spot into the appropriate wells of the microtiter strip plate.

2. Add 200 µL of eluting buffer to each well.
3. Cover plate, hand-shake (gently) for 30 seconds, and incubate overnight at room temperature.*
   * Be certain all blood spots are submerged in eluting buffer.
4. Aspirate contents of all wells. Wash 3 times with 300 or 200 µL wash buffer and aspirate or "flick" plate to dryness after each wash.
5. Add 100 µL of diluted enzyme-conjugate to each well.
6. Cover plate, mix gently by hand (30 seconds), and incubate for one hour at room temperature.
7. Aspirate contents of all wells or "flick" plate to dryness.
8. Wash 3 times with 300 µL wash buffer and aspirate or "flick" plate to dryness after each wash.
9. Add 100 µL of fresh substrate to each well.
10. Incubate 15 minutes at room temperature.
11. Add 100 µL of stopping solution to each well and shake for 10 seconds - horizontally by hand.
12. Read absorbance at 450 nm and plot on semi-log graph paper (Abs. vs. log dose in ng/mL). Absorbance can be read anytime after addition of stop solution up to a maximum of 60 minutes.

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PROTOCOL

Wells	Sample	Eluting Buffer		Wash Buffer	Enzyme Conjugate		Wash Buffer	Color Substrate		Stopping Solution	Read
A1 , B1	Calibrator 1	200 µL	Incubate overnight at room temperature	3 times, 200 µL or 300 µL	100 µL	Incubate 1 hour at room temperature	3 times, 200 µL or 300µL	100 µL	Incubate 15 minutes at room temperature	100 µL	Read Abs. at 450 nm
C1 , D1	Calibrator 2	200 µL			100 µL			100 µL		100 µL	
E1 , F1	Calibrator 3	200 µL			100 µL			100 µL		100 µL	
G1 , H1	Calibrator 4	200 µL			100 µL			100 µL		100 µL	
A2 , B2	Calibrator 5	200 µL			100 µL			100 µL		100 µL	
C2 , D2	Calibrator 6	200 µL			100 µL			100 µL		100 µL	
E2 , F2	Calibrator 7	200 µL			100 µL			100 µL		100 µL	
G2 , H2	Level I	200 µL			100 µL			100 µL		100 µL	
A3 , B3	Level II	200 µL			100 µL			100 µL		100 µL	
C3 , D3	Level II	200 µL			100 µL			100 µL		100 µL	
E3 , F3	Unknown	200 µL			100 µL			100 µL		100 µL	

CALCULATIONS AND INTERPRETATIONS

A. Read sample absorbances directly off curve as ng/mL serum. A sample assay and a standard curve is provided in Section 13.

B. Samples with Trypsin levels beyond the highest standard should be reported as “greater than.....”.

ANTIBODY CONSTITUENTS

The Neonate Trypsin ELISA kit uses a polyclonal "capture" antibody configuration on the microwell plate and a complementary HRP-labeled monoclonal antibody as tracer. The monoclonal antibody is produced from mice immunized with human Trypsin. The polyclonal antibody was raised in Trypsin immunized rabbits.

REFERENCES/LiteraTure

1. Adrian, T.E. et al., Clin. Chem. Acta. 97:205, 1979.
2. Andrulli, A., et al., Dig. Dis. Sci. 26:532, 1981.
3. Lake-Bakaar, G et al., Lancet i:66, 1977.
4. Gamble, D. R. et al., J. Clin. Pathol, 32:897, 1979.
5. Elias, E. et al., Lancet i:66, 1977.
6. Adrian, T. E, et al., Gut. 19: A446, 1978.
7. Healey, A. F., Watson D., Clin. Chem. 29:2011, 1983.
8. Kirby, L. T. et al., Clin. Chem. 27: 678, 1981.
9. Crossley, J. R. et al., Lancet, i March 3, 472, 1979.
10. Kirby, L., et al., 29:1559, 1983.
11. Travis, J. C., Unpublished observations, 1985.
12. Fahrenkrug, J. et al., Clin. Chem. 27:1655, 1981.
13. Koehn, H. D., A. Mostbeck, Clin. Chem 27:502, 1981.
14. Travis, J. C., et al., Clin. Chem., 25,735, 1979.
15. Coury, A. J., et al., Clin, Chem., 29:1593, 1983.
16. Scwachwon, H., Mohmoodian, A., Clin. Chem., 25:158, 1979.
17. Thompson, L. S., Clin. Chem. News, pg 8, 1984.
18. Ryley, H. C., J. Clin, Pathol., 34:179, 1981.
19. Margolies, R., Boat, T.F., Pediatr. Res., 17:931, 1983.
20. Quissel, D. O., et al., Pediatr. Res., 17:899, 1983.
21. Reiter, E. O., et al., Clin. Endocrinol., 16:127
22. Malvano, R. et al., Scand J. Gastroenterol., Supp. 62, 150:3, 1980.
23. Travis, J. C., "Poster" III International Conference on Newborn Screening for Cystic Fibrosis, Oct. 5-6, 1988, CAEN, France.
24. Travis, J. C., "Poster" 7 th National Neonatal Screening Symposium, Nov. 15-19, 1989, New Orleans, LA.
25. Travis, J.C., "Poster" IV th International Conference on Newborn Screening for Cystic Fibrosis, Oct. 8-9, 1990, Estes Park, CO.

26. Travis, J. C., "Poster 8 th International Neonatal Screening Symposium and Inaugural Meeting of the International Society for Neonatal Screening, Leura, N.S.W., Australia, Nov. 11-15, 1991.
27. Travis, J. C., "Poster" 9th National Neonatal Screening Symposium, Raleigh, NC, April 7-11, 1992.