REFERENCES

1. Anne M. Lewis, Christine Waterhouse, and Laurence S. Jacobs. (1980). Whole-Blood and Plasma Amino Acid Analysis: Gas-Liquid and CationExchange Chromatography Compared. CLIN. CHEM. 26/2, 271-276
2. Hortin GL (2012). In: Burtis CA ed. Amino acids, peptides, and proteins. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th ed. Philadelphia, PA: Elsevier Saunders; 2012:chap 21
3. Naylor, E.W. (1993) in Laboratory Methods for Neonatal Screening (Therrell, B.L. Jnr. ed.) Apha p115
4. Snyderman, S.E., Norton, P.M., Roithan, E., and Holt, L.E. Jnr. (1964) Pediatrics 34: 454
5. Naylor, E.W. (1980) in Neonatal Screening for Inborn Errors of Metabolism (Bickel, H., Guthrie, R. and Hammersen, G., eds.) Berlin, Springer
6. Slazyk, W.E. & Hannon, W.H. (1993) Quality Assurance in the Newborn Screening Laboratory in Laboratory Methods for Newborn Screening (Therrell, B.L. ed.) Am. Pub. Health Assoc. 1015 Fifteenth Street N.W. Washington D.C. 20005 p23.
7. Westgard, J.O. & Klee, G.G. (1994) Quality Assurance in Tietz Textbook of Clinical Chemistry (Burtis, C.A. & Ashwood, R. eds.) 3RD Edition, W.B. Saunders, Philadelphia, PA. p548

2020-03-09

RUO

Calbiotech, Inc.
1935 Cordell Ct., El Cajon, CA 92020 USA
Tel (619) 660-6162 | Fax (619) 660-6970 | Web www.calbiotech.com

CALBIOTECH
A Life Science Company

Neonatal Galactose ELISA

Catalog No.: GL345N (96 Tests)

INTENDED USE

The Calbiotech Galactose Screening Assay is an enzymatic colorimetric end-point method for the determination of total D-Galactose in dried blood spot specimens taken from newborn human infants as part of a newborn screening program.

PRINCIPLE OF THE TEST

The Calbiotech Galactose Kit uses trichloroacetic acid (TCA) to extract Galactose from dried blood spot samples. After extraction, the eluted sample is combined with the enzyme reagents Galactose Dehydrogenase and Alkaline Phosphatase . These enzyme reagents catalyzes the NAD-dependent oxidative reaction of Galactose to D-galactono-1,4-lactone + NADH + H+. The NADH produced reacts with a color reagent in which a tetrazolium salt gets reduced producing a distinct color.

MATERIALS PROVIDED	96 Tests
1. Elution Microplate	12x8x1
2. Reaction Microplate	12x8x1
3. Elution Reagent	12 mL
4. GAL Calibrators: 6 Calibrators	0.5mL ea
5. GAL Controls, 3 levels Dried Blood Spots per card	6 DBS
6. Enzyme: 1 vial (ready to use)	240 uL
7. Cofactor: 1 bottle (Lyophilized)	5 mL
8. Cofactor Diluent	6 mL
9. Reaction buffer: 1 bottle (ready to use)	8 mL
10. Color Reagent: 1 bottle (ready to use)	9 mL

.

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2-8ºC.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.
4. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
5. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
6. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
7. All blood samples of human origin should be regarded as a potential Biohazard. Handle all blood samples as if capable of transmitting Hepatitis and HIV
8. Reagents, standards and Controls contain sodium azide (NaN_3) and ProClin as antimicrobial preservatives. Users should be aware of their toxic properties if absorbed or ingested. Disposal of these reagents should be accompanied by copious flushing with water to avoid accumulation of explosive salts in plumbing systems.
9. Do NOT keep or use the reconstituted Enzyme, Coenzyme, or the combined Enzyme-Coenzyme working solution for any longer than the specified periods of time.
10. All the kit components used in an assay must be from the same kit.
11. Kit components and test specimens should be at room temperature before starting the assay.
12. Do not use any reagents or solutions that have become cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING

Follow the guidelines in the NCCLS publication LA4T7 for collecting blood samples in the neonatal screening program, copies of which can be obtained from: NCCLS, 771 E. Lancaster Ave, Villanova, PA 19085. Use WHATMAN type 903 filter paper. For samples screening for CAH, collect samples 3 to 5 days after birth. Use disposable lancets with tips less than 2.5 mm to prick the medial or lateral sides of the bottom of the heel. Allow a drop of blood to form with sufficient volume to fill a 5/8-inch diameter spot on filter paper. Gently touch the drop of blood with the filter paper. **DO NOT PRESS AGAINST THE SKIN. DO NOT TOUCH SPOTTED AREA**. Suspend spotted papers horizontally and allow drying at room temperature for a minimum of 3 hours. Avoid spots touching other surfaces and keep away from direct light. The samples should be transported to the laboratory within 24 hours after collection in appropriate storage container. The laboratory should store the specimens at 2-8 °C protected from moisture and direct light.
The dried blood spots are stable for at least 3 weeks at 2-8 °C protected from light and moisture. Reject samples with the following conditions:

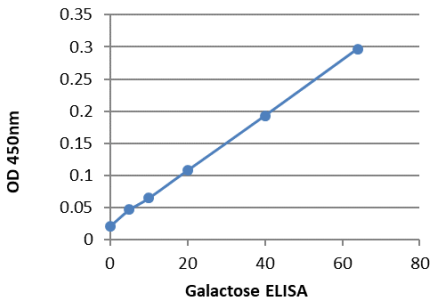
1. Specimens not collected on WHATMAN type 903 filter paper.
2. Blood spots not completely saturated on both sides.
3. Blood spots with appearance of caking or clotting.

REAGENT PREPARATION

1. **Wash Buffer**: Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475ml of distilled water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

1. Equilibrate all reagents to room temperature.
2. Take a clean 96-well elution microplate and add dried blood spots (2 discs of 1/8inch diameter) of controls and samples to corresponding well.
3. Add 100 µl of Elution Buffer to each well, mix the contents and place the plate on a plate shaker.
4. Incubate 30 minutes at room temperature on shaker at 900rpm (cover from light).
5. Reconstitute the lyophilized Cofactor with 5ml of Cofactor Diluent (protect from light).
6. Mix **60**µl of Reaction buffer, **40**µl of Cofactor and **2** µl of enzyme per test. **Note:** Calculate the needed volume including standards and controls to make a master mix for all the wells. This mixture is stable for 5 hours.
7. Transfer 40 µl of the eluted controls and samples to a Reaction Microplate, also transfer 40 µl of each standard (liquid, ready to use) at the corresponding wells.
8. Add 100 µl per well of the mixture prepared in step 7. Incubate 30 minutes at room temperature shaking at 900rpm (cover from light).
9. Add 80 µl of Color Reagent ready to use, per well. Mix well and incubate 5-10 min on bench. Longer incubation time can contribute to background increase.
10. Measure the absorbance at 450 nm, endpoint mode, single measurement. Calculate the slope and the sample values.



CALCULATION OF RESULTS

A standard curve is constructed as follows:

1. Calculate the average absorbance values for each set of standards and patient samples.
2. To construct the standard curve, plot the mean absorbance of each Galactose standards (vertical axis) against its concentration in mg/dl (horizontal axis).
3. Draw the best-fit curve through the plotted points.
4. Read the absorbance for each unknown sample from the curve to determine the corresponding concentration of Galactose.

Example of a Typical Standard Curve

	OD450nm	mg/dl
Std 1	0.021	0
Std 2	0.047	5
Std 3	0.065	10
Std 4	0.108	20
Std 5	0.193	40
Std 6	0.297	64

EXPECTED VALUES

We recommend each laboratory to establish its own normal ranges, for the population it serves. Until then, literature values may be used as guidelines: < 10 mg/dl

REPORTABLE RANGE: Analytical Range = 5 - 64 mg/dl Samples that fall within the calibration curve should be reported as such.