9051 Mira Mesa Blvd, #910094
San Diego, CA 92191
Tel: 760-390-3989

Neonatal PKU ELISA Kit

Name

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INTENDED USE

The Calbiotech Phenylalanine Kit is an Enzyme Assay (EA) for the quantitative measurement of Phenylalanine concentrations in dried blood spot samples that have been collected onto Schleicher & Schuell (S&S®) 903™ Specimen Collection Paper. This Kit is particularly suitable for use in a newborn screening program.

PRINCIPLE OF THE ASSAY

The Calbiotech Phenylalanine Kit uses trichloroacetic acid (TCA) to extract Phenylalanine from dried blood spot samples. After extraction, the eluted sample is combined with the enzyme reagent Phenylalanine dehydrogenase. This enzyme reagent catalyzes the NAD-dependent oxidative deamination of Phenylalanine to phenyl pyruvate and ammonia. 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. Calibrators 6	0.5ml
5. Controls-3 levels Dried Blood Spots	6 DBS
6. Phenylalanine Dh Enzyme: 1 vial (ready to use)	120 ul
7. Cofactor: 1 bottle (Lyophilized)	5 ml
8. Cofactor Diluent	6 ml
9. Reaction buffer: 1 bottle	8 ml

(ready to use)	
10. Color Reagent: 1 bottle (ready to use)	9 ml

MATERIALS NOT PROVIDED

1/8” inch hole punch

Shaker capable of fixed speed

Distilled or deionized water

Precision pipettes

Disposable pipette tips

Reader capable of reading absorbance at 450 nm

Absorbance paper or paper towel

Graph paper

ASSAY PRECAUTIONS

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

Reagents, Standards and Controls

contain sodium azide (NaN3) 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.

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.

All the Kit components used in an assay must be from the same kit.

	O450nm	[mg/dl]
Std 1	0.0682	0.00
Std 2	0.0880	1.00
Std 3	0.1080	2.00
Std 4	0.1450	4.00
Std 5	0.2230	8.00
Std 6	0.3670	16.00

Kit components and test specimens should be

at room temperature before starting the assay.

	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: Specimens not collected on WHATMAN type 903 filter paper. Blood spots not completely saturated on both sides. Blood spots with appearance of caking or clotting.
ASSAY PROCEDURE:	Equilibrate all reagents to room temperature. 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. Add 100 µl of Elution Buffer to each well, mix the contents and place the plate on a plate shaker. Incubate 30 minutes at room temperature on shaker at 900rpm (cover from light).

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	Reconstitute the lyophilized Cofactor with 5ml of Cofactor Diluent (protect from light). Mix 60µl of Reaction buffer, 40µl of Cofactor and 1 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. 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. Add 100 µl per well of the mixture prepared in step 7. Incubate 30 minutes at room temperature shaking at 900rpm (cover from light). 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. 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: Calculate the average absorbance values for each set of standards and patient samples. To construct the standard curve, plot the mean absorbance of each Phenylalanine standards (vertical axis) against its concentration in mg/dl (horizontal axis). Draw the best-fit curve through the plotted points. Read the absorbance for each unknown sample from the curve to determine the corresponding concentration of Phenylalanine.
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: Phenylalanine normal range REPORTABLE RANGE: Analytical Range = 1 - 16 mg/dl Samples that fall within the calibration curve should be reported as such. PERFORMANCE CHARACTERSTICS Sensitivity

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The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 24 times in the same run.

Serum	No. of Replicates	Mean (mg/dl)	Standard Deviation	Mean + 2SD (Sensitivity)
Zero Standard	24	0.0582	0.0610	0.1802

Precision

Intra-Assay

Serum	No. of Replicates	Mean (mg/dl)	Standard Deviation	Coefficient of Variation (%)
1	16	1.83	0.087	4.80
2	16	2.45	0.0867	3.53
3	16	5.16	0.106	2.05

Precision

Inter-assay

Serum	No. of Replicates	Mean (mg/dl)	Standard Deviation	Coefficient of Variation (%)
1	20	1.91	0.080	4.21
2	20	2.44	0.093	3.80
3	20	5.05	0.117	2.32

Linearity

Three different patient samples were diluted to ½, 1/4 & 1/8. Phenylalanine values were calculated, and results were corrected with the dilution factor.

	Original Value	Percentage of Recovery	Percentage of Recovery	Percentage of Recovery
Serum	mg/dl	1/2	1/4	1/8
1	15.1	99.4	101	98.8

2	16	93.3	96.2	93
3	16.5	101.3	107.9	104.6

REFERENCES

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