

## NEO-PKU

Quantitative enzymatic assay of Phenylalanine in dry blood spot  
Catalogue No.: PT-Neo-PKU-96

### PISHTAZ TEB DIAGNOSTICS

#### Introduction

Phenylketonuria, called PKU for short, is an inherited disorder that is characterized by inability of the body to utilize the essential amino acid, phenylalanine. Unless the condition is detected and treatment is initiated soon after birth, this hereditary biochemical abnormality, prevents normal brain development and usually results in mental retardation

The PKU-Neo kit is specially designed to be used in phenylalanine assay on the dry blood spot (DBS) collected from newborn's heel during the first 48 hours of birth.

#### Principle of the assay

The figure 1 demonstrate that the assay is based on enzymatic determination of phenylalanine, using the phenylalanine dehydrogenase ( Phe-DH ) and an intermediate electron acceptor system to make it applicable for colorimetric measurement

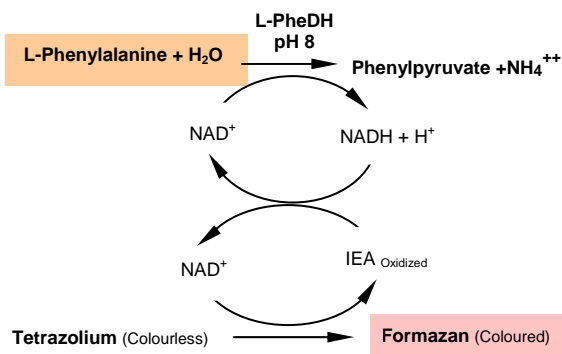


Fig. 1- Reaction scheme of the enzymatic Phenylalanine determination.

#### Sample collection and storage

Within 3 days after birth, collect a blood sample from the heel of the infant.

- 1- Clean the heel of the infant with soap and water. Wipe area dry. Use alcohol (70% isopropanol) on the area and air dry.
- 2- With a lancet (2.4 mm in length), prick the heel once and wipe away the initial drop of blood. After another drop is formed, use the sample collection card to collect the infant's blood on the card. Do this by gently pressing the drop of blood in to the center of the pre-printed circle on the sample collection card (S&S 903). Do not tear or scratch the filter paper surface. To avoid hemolysis and dilution of the blood sample do not exert excessive pressure during collection.
- 3- Let sample card air dry, for no less than 3 hours at room temperature (20-25°C). Place card in a clean area and away from direct sunlight and heat.
- 4- Within 24 hours, place each sample in individual paper envelope. Place in a moisture-proof bag at 2-8°C for short-term storage and -20°C for long-term storage.

#### Reagents Provided with Test Kit

Extraction plate	1× 96 wells
Test plate	1× 96 wells
Plastic cover	1 sheet
Reagent # 1- Extraction buffer	1× 20 ml
Reagent # 2- Substrate solution	1× 6 ml
Reagent # 3- Buffered enzyme solution	1× 6 ml
Reagent # 4- Chromogen solution	1× 6 ml

Phenylalanine standard and control spots (2 sheets) prepared on filter paper (S&S 903) containing known amounts of phenylalanine diluted in washed human blood.

#### Materials required but not supplied

- Microplate reader (490 nm filter)
  - Water bath (90-95°C)
  - Hole-puncher (5mm).
- (This item can be purchased from Pishtaz Teb Zaman)
- Plate shaker

#### Assay procedure

All reagents should be brought to room temperature (18-25°C) before use:

- 1- Dispense standards, samples and controls by punching 5mm blood spotted filter disk in the

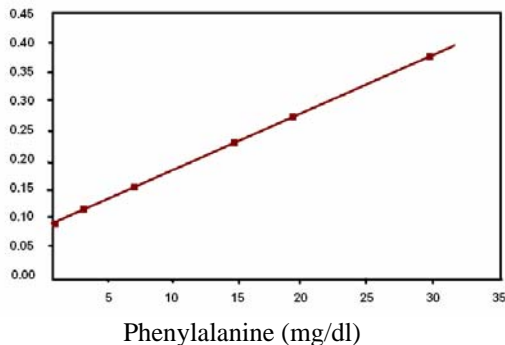
## Neo-PKU Kit

correct wells of Extraction plate (Preferable do it in duplicate).

- 2- Place the plate on stand over 90-95°C hot water vapor (water bath) for 4-5 minutes in order to fix the blood on paper .
- 3- Add 150 µl of extraction buffer in to each well. Be sure that the whole disk is soaked in buffer and no bubble is produced in wells.
- 4- Cover the plate with plastic cover and shake slowly for 90 minutes at room temperature. **(Keep plastic cover clean for the whole procedure).**
- 5- Transfer 100 µl of the extract from each well in to corresponding wells of the **Test plate.**
- 6- Mix equal portions of reagent # 2, #3 and #4 as the volume needed and mix them carefully. **The mixture is stable only for 5 minutes.**
- 7- Add 150 µl of this mixture in to each well.
- 8- Cover the plate and shake it slowly for 60 minutes at room temperature.
- 9- Read the absorbance at 490 nm within 10 minutes.

### Calculation of results

- Calculate the mean absorbancy value for each set of standards, controls and samples.
- Construct a standard curve by plotting the absorbancy obtained from each reference standard against its concentration of Phenylalanine on linear graph paper.
- Determine the Phenylalanine concentration for each sample on standard curve. Results of a typical standard run of a neonatal Phenylalanine are shown below.



### Performance characteristics

- 1- **Within-run precision** was determined by replicate determination of two different whole blood samples of known concentration of Phenylalanine in one assay.

Sample	1	2
Replicates	10	10
Mean Conc.(mg/dl)	1.0	6.0
S. D	0.08	0.42
%CV	11.0	7.6

2- **Between-run precision** was determined by replicate measurements of two different test samples of known concentration in 8 different assays.

Sample	1	2
Replicates	8	8
Mean Conc.(mg/dl)	1.0	6.0
S. D	0.14	0.65
%CV	12.7	9.8

3- **Sensitivity:** the minimal detectable concentration of phenylalanine was determined by adding two values of standard deviation to the mean of optical density of 12 replicates of zero standard and calculating the corresponding concentration from the standard curve. The minimum detectability was found 0.5 mg/dl.

4- **Specificity:** this kit exhibits no significant detectable cross-reactivity with other amino acids.

5- **Recovery:** was determined by adding different concentrations of phenylalanine to whole blood samples. Recovery was more than 90%.

Added (mg/dl)	Measured (mg/dl)	Recovery %
5	4.55	91.0 %
10	9.35	93.5 %
20	18.9	94.5 %

### Reference Value:

Phenylalanine concentration of the whole blood collected from 250 healthy newborns aged 24 – 36 hours was as the following table:

Phenylalanine ( mg/dl )	
max	3.9
min	1.2
mean	1.8
SD	0.1

### References:

- Hammond , K.B.et al ., N ENGL J Med 325:303 , 1990
- Yamaguchi , A . et al ., Screening 1 : 49 , 1992
- Wendel , U . et al ., Clin Chem. Acta 192 : 165 , 1990
- Lww , C., Lab Med 24 ( 5 ) : 301 , 1990