



PKU

Screening assay

**Enzyme colorimetric assay
for the quantitative determination
of Phenylalanine levels
in newborns**



ZenTech

ORDERING INFORMATION

Code: **E-HN-500**

Package Size : 500 tests/kit

Code: **E-HN-2000**

Package Size : 2000 tests/kit



Indications

- Quantitative measurement of Phenylalanine concentrations in dried blood spot collected onto Schleicher & Schuell 903 or 2992 specimen collection paper.
- Suitable for use in a neonatal screening program to measure Phenylalanine concentrations in newborns infants as an aid to the detection of Phenylketonuria.

Features

- Convenient transport and good stability of the samples
- Accurate, sensitive, rapid and specific assay

Kit contents

Reagents	500 tests	Quantity	2000 tests
Enzyme	4 vials x 5 ml		4 vials x 20 ml
Coenzyme	4 vials x 5 ml		4 vials x 20 ml
Dilution buffer	1 vial x 10.5 ml		1 vial x 42 ml
Colour Reagent	1 bottle x 43 ml		1 bottle x 175 ml
Colour Reagent Booster	1 vial x 4.3 ml		1 vial x 17.5ml
One set of standards / controls of Phenylalanine			



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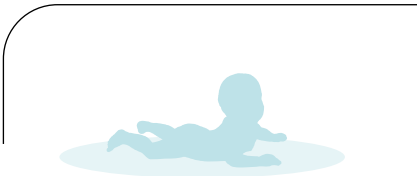
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ZenTech

NEONATAL SCREENING



PKU

Screening assay



Simple assay procedure

1. Take a **clean** 96-well (preferably **U** bottom) microplate (**elution microplate**).
2. Add one disk cut from a dried blood spot (**4.7 mm or 2 X 3.2 mm diameter**) per well. Remember to add **controls, standards and one blank well**.
3. Warm up all reagents (except the color reagent) to room temperature.
4. Add 100 μ l of Elution Buffer (TCA 3%) in each well, **mix well** the contents of each well and place the plate on a plate shaker.
5. Wait **30 minutes** at room temperature (20-26 $^{\circ}$ C).
6. While waiting reconstitute one Enzyme vial and one Coenzyme vial with **20 ml distilled water each**. Stable for at least one week refrigerated. Mix **2 parts** of Enzyme solution with **2 parts** of Coenzyme solution and **1 part** of Dilution buffer. You need 100 μ l of this Enzyme-Coenzyme-Dilution buffer mixture for each sample. Please note that you should only mix the quantity you need for the day's run. The Enzyme-Coenzyme mixture **should be discarded if not used within 5 hours**. The following table gives the volumes required from each of the three components to run specific number of tests (**volumes in ml**). **We highly recommend the addition of the Dilution buffer just before using the mixture.**

# tests	Enzyme (ml)	Coenzyme (ml)	Dilution buffer (ml)	Total Volume (ml)
50	2	2	1	5
100	4	4	2	10
150	6	6	3	15
200	8	8	4	20
300	12	12	6	30
400	16	16	8	40
500	20	20	10	50

7. Transfer **40 μ l** of the TCA eluant in a new microplate in the corresponding wells. Add 100 μ l of the mixture prepared in step 6 per well. Mix well, avoiding the formation of foam. Wait for **30 minutes** at room temperature (20-26 $^{\circ}$ C).
8. Take the Color Reagent and the Color Reagent Booster out of the refrigerator, mix one part of Color Reagent Booster with 10 parts color reagent just before using it. Do not pre-warm the mixture. Return the original bottles back to the refrigerator the soonest possible. Avoid exposure to light. Prepare only the quantity you will need for the day.
9. Add **80 μ l** of Color Reagent mixture per well. **Mix well** avoiding the formation of foam.
10. Wait for **10 minutes** and measure the microplate at **550 nm**, endpoint mode, single measurement. **There is no need to wait longer than 15 minutes.**
11. Calculate the slope and the sample values.

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