Manual

Kryptopyrrole Kit

For the in vitro determination of Kryptopyrrole in urine

For research use only

Distributed in the US and Canada by:

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REF **K7823**

Σ 40

+ 8 °C
+ 2 °C

RUO

1. INTENDED USE

The described assay is intended for the colorimetric determination of kryptopyrrole in urine. For research use only.

2. INTRODUCTION

Kryptopyrroluria is a genetically determined enzymatic disorder of the hem metabolism, which is essentially compensated under normal conditions. However, under stress conditions, there is an insufficient compensation and the disorder is manifested in the form of several different disease symptoms. Kryptopyrroluria can be diagnosed with high reliability by detection of abnormal increased pyrrole excretion in urine.

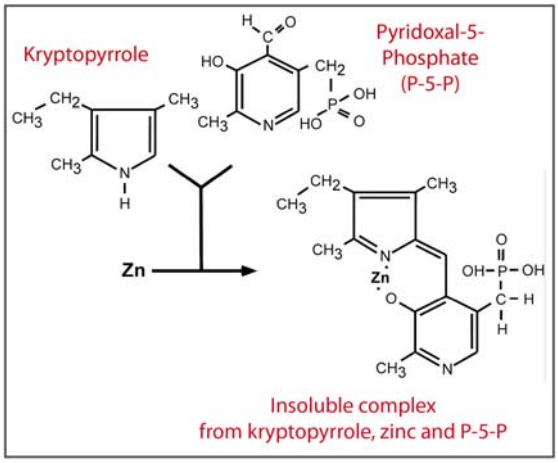
Kryptopyrrole, 2,4 dimethyl-3-ethylpyrrole, is a byproduct of disordered hemoglobin synthesis. It has been found out that circulating kryptopyrrole forms a Schiff's base with the aldehyde group of pyridoxal-5'-phosphate (P-5-P), the active vitamin B_6 form in the blood, whereby an additional zinc binding results in formation of an insoluble kryptopyrrole – zinc - vitamin B_6 - complex. Elevated excretion of kryptopyrrole in the urine is associated with an enhanced vitamin B_6 and zinc excretion and as a result in a B_6 and zinc deficiency in the blood.

Kryptopyrroluria is involved in behavior and emotional disorders. Increased kryptopyrrole values were found in children and juveniles with ADHS (Attention Deficit Hyperactivity Syndrome), as well as in children with disturbed short-term memory, learning difficulties, dyslexia and stress-intolerance. Positive therapy experiences were made with substitution of zinc, vitamin B_6 and manganese.

Our colorimetric test is designed for determination of urinary kryptopyrrole. Urine specimen collection and sample transport are very important for proper test results. Special urine test tubes for kryptopyrrole determination can be ordered in addition to the test kit.

Indications

- Behavior disorders
- Autism, Asperger´s
- ADHD, ADD
- Depression
- Bipolar disorders
- Assaultive/aggressive/violent behavior
- Schizophrenia
- Irritability



- Poor tolerance of physical and emotional stress
- Anger
- Episodes
- Poor memory
- Agitation
- Poor dream recall
- Sensitivity to light and sound

3. MATERIAL SUPPLIED

Catalogue No	Content	Kit Components	Quantity
K7823FR	REAG	Color developing reagent	1 x 30 ml
K7823LA	SOLA	Solution A	1 x 40 ml
K7823LB	SOLB	Solution B **(Warning: concentrated H_2SO_4!)**	1 x 2 ml
K7823ST	STDKONZ	Standard concentrate	1 x vial
K7823TM	DRY	Drying agent	1 x 15 g
K7823VP	DIL	Standard dilution solution	1 x vial

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- 1N Hydrochloric acid
- Dichloromethane
- Bidistilled water (aqua bidist.)
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Centrifuge capable of 3000 rpm
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Spectral photometer, 540 nm
- Ice bath
- Quartz cuvette

5. PREPARATION AND STORAGE OF REAGENTS

- Dissolve **REAG** (color developing reagent) immediately before test performance with 28.5 ml of SOL A (Solution A). Cool the prepared solution in an ice bath and then add drop-wise, slowly and carefully 1.5 ml of SOL B (Solution B) **(Warning: Heat development!).** This solution is stable at 2-8°C for 2 days.
- Dissolve **DIL** (Standard dilution solution) in exactly 30 ml of bidistilled water immediately before use. Ensure that any remaining powder on the cap and screw thread will be dissolved. It is essential to protect the solution from light.
- Dissolve **STDKONZ** (Standard concentrate) with 45 µl of SOL A (Solution A). For this, add SOL A (Solution A) into the glass vial and shake it for 2-3 minutes. Thereafter, add 4.95 ml of the prepared DIL (Standard dilution solution). The concentration of the obtained standard concentrate is 1 mg/ml.
- Using 50 µl of the dissolved standard concentrate (1 mg/ml) and 4.95 ml of the prepared DIL (Standard dilution solution), prepare diluted standard solution with a concentration of 10 µg/ml.
- Prepare a standard series of the above diluted standard solution (10 µg/ml) according to the following table:

Standard concentration	Standard dilution solution	Diluted standard solution (10 µg/ml)
STD 0 ng/ml	2000 µl	no
STD 100 ng/ml	1980 µl	+ 20 µl
STD 250 ng/ml	1950 µl	+ 50 µl
STD 500 ng/ml	1900 µl	+ 100 µl
STD 1500 ng/ml	1700 µl	+ 300 µl

- All test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

6. SAMPLES

Urine is used as a sample material.

7. ASSAY PROCEDURE

Test procedure

1.	Determine the **pH-value of the urine** and, if necessary, adjust it with 1N Hydrochloric acid to pH 3 – 4.
2.	Transfer **2 ml of the acidified urine** into a glass reaction tube and extract it with 4 ml dichloromethane by shaking for app. 2 minutes. Then centrifuge for 5 minutes at 3000 rpm and discard completely the aqueous supernatant.
3.	On the tip of a spatula, take a small quantity of **DRY** (drying agent) and add it to the o**rganic phase** in order to bind the remaining water. Allow the DRY (drying agent) to sedimentate for about 2 minutes.
4.	Transfer with a pipette **2 ml of the organic phase** in a clean reaction glass and add 0.5 ml of the REAG (dissolved color developing reagent).
5.	**After 30 minutes, absorption** is measured in a quartz cuvette on a spectral photometer at **540 nm**.
6.	**Standards** are not extracted, only 0.5 ml of REAG (dissolved color developing reagent) is added, and measured like the samples at 540 nm.

8. RESULTS

For evaluation of the sample results, we recommend to produce a standard curve by drawing a point-to-point curve between the obtained values of the standards. **The sample results are multiplied by 2.25**. Please consider eventual additional dilution factors due to acidifying with hydrochloric acid.

9. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

10. PRECAUTIONS

- For research use only.
- Quality control guidelines should be followed.
- SOL B (Solution B) contains a strong acid and must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.

11. TECHNICAL HINTS

- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.

12. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- All reagents in the kit package are for research diagnostic use only.
- Guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

13. REFERENCES

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Used symbols:

Temperature limitation

REF Catalogue Number

RUO For research use only

Contains sufficient for <n> tests

Manufacturer

Use by

LOT Lot number