HYDROXYPROLINE NEW

Colorimetric determination on urine

10 x 3 tests **REF CM08-30T**

PRINCIPLE

Hydroxyproline in urine is in a free state (3%) and bound to peptides (97%). It is liberated by hydrolysis. Oxidized to pyrrole, with Ehrlich reagent it forms a red compound which can be measured photometrically.

DIAGNOSTIC IMPLICATIONS

Hydroxyproline is a major component of the protein collagen. Excretion of urinary hydroxyproline is a useful parameter to evaluate catabolic conditions of bone and connective tissues (bone metastasis, Paget disease, osteolytic process, etc.).

REAGENTS AND MATERIALS

Kit components:	CM08-30T	Quantity
(*) **REAGENT 1/2** **REAGENT 1** (pre dosed powder) Oxidant, contained in injection cap	**CM08-30TR1**	**10x150 mg**
REAGENT 2 Buffer pH 6.0 contained in the vial	**CM08-30TR2**	**10x2.5 ml**
(*) **REAGENT 3** (pre dosed powder) Chromogenous	**CM08-30TR3**	**10x200 mg**
(*) **REAGENT 4** Alkalinizer	**CM08-30TR4**	**1x35 ml**
(*) **REAGENT 5** Hydroalcoholic mixture	**CM08-30TR5**	**1x70 ml**
(*) **REAGENT 6** Perchloric acid	**CM08-30TR6**	**1x15 ml**
HYDROXYPROLINE STANDARD 250 mg/L	**CM08-30TS**	**1x2 ml**

STABILITY: stored at 4°C in a closed bottle, reagents are stable up to the expiration date.

REAGENT NOT INCLUDED WITH THE KIT

Concentrated hydrochloric acid f.a.

REQUIRED EQUIPMENT

Spectrophotometer or filter photometer (560 nm), centrifuge, thermostatic bath at 60°C, 102°C or at 121°C.

PREPARATION OF WORKING REAGENTS

REAGENT A (R1/2 + R5)

Remove the yellow capsule. Push the red piston to the bottom so the powder inside the cap falls into the vial. Remove the cap and pipette 2.5 ml of Reagent 5. Shake until complete dissolution.

STABILITY: **use, at the latest, within 15 minutes after it is prepared.**

REAGENT B (R3 + R5 + R6)

Pipette 4 ml of Reagent 5 and 1.2 ml of Reagent 6 into a vial of Reagent 3, shake until complete dissolution.

STABILITY: **use, at the latest, within 2 hours after it is prepared.**

SAMPLE

24-hour urine

Starting 24 hours before and for the entire period of the collection, the patient must keep a low-collagen or collagen free diet. Avoid meat and its derivatives, fish, ice cream, sweets and any products containing gelatin. Milk, and its derivatives, eggs, vegetable broth, fruit and vegetables are allowed. Collect the 24-hour urine and store in a tightly closed container at 4°C. After collection, measure the volume and note it. Mix carefully and take approximately 10 ml of urine for the assay. The presence of a precipitate does not interfere with the test. If urine is very turbid, centrifuge and use the supernatant.
Note the patient's age, weight and height.

STABILITY: 24 hours at 4°C, at least 2 days at -20°C.

NORDIN TEST

Nordin test defines the hydroxyproline urinary elimination regardless of the diet, the renal function level, the weight and body mass.

Hydroxyproline is evaluated on urines of the second fasting in the morning and compared to the creatinine elimination on the same urine sample.

24-hour urine

At 11 p.m. of the day before the test, the patient must be fasting (no food, no drink, no water) and must not smoke until 9 a.m. of the following day.
At 7 a.m. of the test day, the patient must empty their bladder completely. Discard this urine. The patient should drink 250 ml of distilled water. At 9 a.m. collect the 2-hour urine and take a sample.

CALCULATION AND REFERENCE VALUES FOR NORDIN TEST

Determine the hydroxyproline concentration and compare it to the creatinine concentration in the sample:

[Hydroxyp. (mg/dl) / creat. (mg/dl)] x 1000=mg hydroxyp./g creatinine

Reference values:
8-20 mg iydroxypr. / g creat. (5-17 mmol hydroxyp. / mol creatinine)

MANUAL ASSAY PROCEDURE

Wavelength:	560 nm
Optical path:	1 cm
Reading:	against blank
Temperature:	102°C (121°C) and 60°C
Method:	colorimetric

NOTE: it is advisable to use HYDROXYPROLINE Extra kit until the operator has a good knowledge of the method.
Pipette into hydrolysis tubes in pirex glassware labeled as follows:

	Sample
Sample	2.0 ml
Concentrated HCl	2.0 ml

WARNING: close tightly the screw cap of collection vials and make sure they are perfectly sealed, to prevent any vapor from leaking during the hydrolysis. This may cause a higher concentration in the sample and lead to possible false positive results.

Incubate the tubes for approx. 16 hours (overnight) at 102°C or for 5 hours at 121°C. Cool the hydrolysis tubes under running water, centrifuge and pipette into 10 ml plastic tubes labeled as follows:
S: sample; B: blank reagent; H/S: hydroxyproline standard.

	S	B	H/S
Supernatant	0.2 ml	---	---
Reagent 4	1.0 ml	----	----
Distilled water	---	1.2 ml	1.2 ml
Hydroxypr. St.	---	---	20 µl
Reagent A	1.5 ml	1.5 ml	1.5 ml

Mix accurately, wait at least 4 minutes and then add:

Reagent B	1.3 ml	1.3 ml	1.3 ml

Close the test tubes and shake. Incubate for 20 minutes at 60°C, cool and read the sample (As) and the hydroxyproline standard (Ash) absorbances at 560 nm against the blank reagent.

CALCULATION

Hydroxyproline (mg/L) = (As/Ahs) x 50

To calculate the 24-hour elimination, multiply the value obtained by the urine volume in liters.

REFERENCE VALUES

Adults 10 - 40 mg of hydroxyproline / liters 24-hour urine
Young and elderly men and women:
values in mg of hydroxypr. / liters 24-hour urine/sq.m. of body surface

AGE	AVERAGE	RANGE
up to 1 year	100	(55 - 220)
1 - 13 years	50	(25 - 80)
over 65 years	12	(5 - 17)

The body surface is calculated by specific tables or nomograms used to evaluate basal metabolism.
Otherwise, Du Bois and Du Bois formula can be used:

body surface in square meters = $H^{0.725} \times W^{0.425} \times 71{,}84 \times 10^{-4}$

with: H = height in cm and W = body weight in kg.

QUALITY CONTROL – CALIBRATION

A quality control program is recommended for all clinical chemistry laboratories.

PERFORMANCE CHARACTERISTICS

Sensitivity:

The test is calibrated to measure with accuracy the normal concentration range.
For considerably higher values, dilute the sample and consider the dilution during calculation.

Within-run precision:

	Level 1	Level 2
Mean (mg/24 hours)	18.5	101
DS	0.15	1.47
CV %	0.81	1.45

Between-run precision:

	Level 1	Level 2
Mean (mg/24 hours)	24	106
DS	0.35	3.98
CV %	1.45	3.75

Correlation: Far kit for hydroxyproline extra shows a correlation coefficient of 0.98 in comparison to another kit available on the market.

Interferences: the presence of chromogens in the sample can cause interferences in hydroxyproline determination. In this case, follow procedure indicated in note 3 below.

DISPOSAL

The product must be used for professional assay only. Dispose of the product according to national/international laws.

WARNINGS AND PRECAUTIONS

REAGENT 1

Advisory: Danger
Hazard Statements:
H302 Harmful if swallowed.
H314 Causes severe skin burns and eyes damage.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

REAGENT 2 e REAGENT 3: Not dangerous.

REAGENT 4

Advisory: Danger
Hazard Statements:
H314 Causes severe skin burns and eyes damage.

REAGENT 5

Advisory: Danger
Hazard Statements:
H225 Highly flammable liquid and vapor.
H319 Causes serious eyes irritation.
H336 May cause drowsiness or dizziness.

REAGENT 6

Advisory: Danger
Hazard Statements:
H271 May cause fire or explosion; very oxidizing.
H314 Causes severe skin burns and eyes damage.

STANDARD: Not dangerous.

NOTES

1. (*) dangerous reagents are marked by an asterisk. Refer to safety data sheet.
2. If the color developed in the reaction is yellowish instead of cyclamen pink, the causes are either insufficient hydrolysis or expired Reagent B. Contact FAR.
3. In case of supposed presence of interfering substances (presence of abnormal chromogenous compounds may lead to higher absorbance values and consequently to an overvaluation of hydroxyproline concentration), follow the procedure up to the addition of Reagent 4. Add 4 ml of butanol to the sample, whirl by vortex, separate the contents by centrifuge, draw and discard the butanol phase. Add Reagent A and after 4 minutes, add another 4 ml of butanol, whirl by vortex, centrifuge, remove the aqueous layer below and follow the procedure. At the end, the color will be brighter.
4. Reaction volumes can be proportionally changed.
5. Disposal of waste according to local laws.

REFERENCES

1. The determination of urinary hydroxyproline (Koevoet et Baars. Clin. Chim. Acta. 25: 39-43. 1969)
2. Routine assay of total urinary hydroxyproline based on resin-catalyzed hydrolysis (Goverde et Veenkamp. Clin. Chim. Acta 41:29-40. 1972)

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IVD

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	read instructions for use

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