17-KETOSTEROIDS

Chromatographic – Colorimetric Determination
of 17-Ketosteroids
in Urine

20 tests **REF KR04-20**

INTENDED USE

Kit for quantitative *in vitro* determination of 17-Ketosteroids in urine.

PRINCIPLE

After acid hydrolysis, 17-ketosteroids are adsorbed on a neutral resin. Interfering substances are separated by washing, 17-ketosteroids are eluted and quantitatively defined by Zimmermann reaction.

REAGENTS AND COLUMNS

Kit components :	REF KR04-20
***REAGENT 1** Hexamethylentetramine	**1 x 1.5 ml**
***REAGENT 2** Alkalizer	**2 x 60 ml**
***REAGENT 3** Alcoholic elutent (store tightly closed)	**1 x 65 ml**
***REAGENT 4** Chromogenous compound (powder)	**1 flaconi**
***REAGENT 5** Alkalizer	**1 x 30 ml**
***REAGENT 6** Extracting solution (store tightly closed)	**1 x 75 ml**
STANDARD Dehydroepiandrosterone 1 g/L	**1 x 1 ml**
COLUMNS Chromatographic columns	**20**

(*) Dangerous reagents are marked by an asterisk. Refer to MSDS

STABILITY: stored at 2-8°C, sealed reagents and materials are stable up to the expiration date on the label.

EQUIPMENT REQUIRED BUT NOT SUPPLIED

Spectrophotometer or filter photometer (520 nm).
Centrifuge, thermostatic bath 25-100°C.

PREPARATION OF REAGENT 4

Add 15 ml of Reagent 3 to the Reagent 4 vial. Shake gently until complete dissolution.
STABILITY: at least 2 months at 2-8°C.

SAMPLE

24-hour urine.
Collect the 24-hour urine in a container with 3-4 ml concentrated hydrochloric acid. Make sure the pH value is between 3 and 6. Mix the urine, measure the volume and store at 2-8°C.
Centrifuge or filter before use.
STABILITY: at least 7 days at 2-8°C.

MANUAL ASSAY PROCEDURE

Wavelength:	520 nm
Optical path	1 cm
Reading:	against blank
Temperature:	4°C or 25°C
Linearity:	180 mg/L
Sensitivity:	0.8 mg/L
Recovery:	90 ± 2 %
C.V.:	0.9 %

PREPARATION OF THE SAMPLE

Pipette into test-tubes labeled as it follows:

	Sample	Blank
Urine	2.5 ml	-----
Distilled water	-----	2.5 ml
Concentrated hydrochloric acid	0.5 ml	0.5 ml
Reagent 1	1 goccia (50 µl)	1 goccia (50 µl)

Mix and put the test-tubes in a hot bain-marie for 10 minutes. Cool the test-tubes with running water.

PREPARATION OF THE COLUMN

Take the upper cap off and snap the bottom tip off. Let the liquid completely flow out.

CHROMATOGRAPHIC SEPARATION

Pour the contents of a SAMPLE test-tube into a column and the contents of the BLANK test-tube into the BLANK COLUMN.Let the liquid completely drain.
Pipette into the columns:

	Sample	Blank Column	
Distilled water	1.0 ml	1.0 ml	
Reagent 2	5.0 ml	5.0 ml	discard the eluate
Distilled water	1.0 ml	1.0 ml	discard the eluate
Reagent 3	2.0 ml	2.0 ml	collect the eluate

Accurately mix the collected eluate.
STABILITY: at least 24 hours at 2-8°C.

COLORIMETRIC REACTION

Pipette into centrifuge tubes with cap labeled a sit follows:

	Sample	Standard	Blank Column	Blank Reagent
SAMPLE Eluate	1.0 ml	----	----	----
Blank Column Eluate	----	----	1.0 ml	----
Standard	----	25 µl	----	----
Reagent 3	----	1.0 ml	----	1.0 ml
Reagent 4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Reagent 5	1.0 ml	1.0 ml	1.0 ml	1.0 ml

Mix accurately. Cap and incubate at 4°C for 60 minutes, or at 25°C for 25 minutes. Then add:

Reagent 6	2.5 ml	2.5 ml	2.5 ml	2.5 ml

Mix thoroughly and centrifuge at 3000 rpm for 5 minutes. Read the sample upper phase (As) Blank column (Abc) and the standard (Astd) absorbencies at 520 nm against the blank.

CALCULATION

17-ketosteroids (mg/L) = (As / Astd) x 20
17-ketosteroids (mg/24 h) = mg 17-ketosteroids/L x L of 24h urine

REFERENCE VALUES

Values expressed in mg/liters of the 24 hour urine

Age in years	Men	Women
0 - 6	0 - 2.3	0 - 2.2
6 - 10	1.1 - 5.6	0.9 - 4.5
11	3.9 - 6.5	2.9 - 8.9
12	4.5 - 7.3	3.3 - 11.5
13	4.8 - 8.0	4.8 - 12.6
14	5.3 - 9.0	4.8 - 13.4
15 - 16	7.6 - 11.0	8.2 - 14.2
17	10.8 - 14.5	10.7 - 15.3
18	10.8 - 17.0	12.0 - 17.0
19	10.9 - 20.0	13.2 - 17.9
20 - 25	15.6 - 23.4	14.0 - 18.8
25 - 40	17.0 - 25.0	11.0 - 19.0
40 - 50	11.4 - 22.0	10.0 - 19.0
50 - 60	9.0 - 18.0	7.3 - 16.8
60 - 70	5.8 - 13.5	5.5 - 13.4
70 - 80	2.9 - 10.0	3.2 - 10.9
oltre 80	2.7 - 8.0	1.8 - 5.8

NOTES

1. Label as BLANK one column. This column will be employed to prepare Blanks and can be used several times. Keep it with resin bed covered with distilled water when not in use.
2. Color development at 4°C is preferred, as results are more reliable for reduced interfering substances formation.
3. Read within 30 minutes after the addition of Reagent 6.
4. If the upper phase is turbid after centrifugation, transfer it into a centrifuge tube containing a spatule full tip of anhydrous sodium sulfate, mix thoroughly and let it deposit.
5. The quantities of the supplied reagent are enough to perform 25 tests (19 samples, 2 standards, 2 reagent blank and 2 blanks).
6. Reaction volumes can be proportionally varied.
7. Centrifuge Hydrolyzed urine if there is a big precipitate. After centrifuge put in the column the upper of Hydrolyzed urine.

REFERENCES

1. R.A. Richardson, Clin.Chim.Acta,50 (1974), 151-152
2. W. Zimmermann, “Z. Physyol. Chem.”, 233, 257 (1935)

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