

# 17-HYDROXY- CORTICOSTEROIDS

Chromatographic – Colorimetric Determination  
of 17-21-Dihydroxy-20-Ketosteroids  
in Urine

40 tests

REF 3614

## INTENDED USE

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

## PRINCIPLE

After the sample treatment, 17-hydroxy-corticosteroids (17-OH CS) are adsorbed on a neutral resin. Interfering substances are separated by washing, 17-OH CS are eluted and quantitatively defined by Porter and Silber reaction.

## REAGENTS AND COLUMNS

Kit components:

\***REAGENT 1** Diluted sulphuric acid **REF 3614**  
**1 x 15 ml**

WARNING: store tightly closed.

\***REAGENT 2** Alkalizer **1 x 15 ml**

WARNING: store tightly closed.

\***REAGENT 3** Diluted sulphuric acid **1 x 105 ml**

\***REAGENT 4/A** Chromogenous compound (pre dosed) **2 vials**

WARNING: STORE REAGENT 4/A AT -20°C

\***REAGENT 4/B** Diluted sulphuric acid **2 x 100 ml**

\***REAGENT 5 Standard** Hydrocortisol 1 g/L **1 x 2 ml**

**REAGENT 6** Adsorbent **1 vial**

**COLUMNS** Chromatographic columns **40**

**MEASURE** **1**

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

STABILITY: stored at 2-8°C (except Reagent 4/A which must be stored at -20°C), sealed reagents and materials are stable up to the expiration date on the label.

## ADDITIONAL REAGENT NOT INCLUDED IN THE KIT

Absolute ethanol for analysis. Centrifuge test-tubes.

## EQUIPMENT REQUIRED BUT NOT SUPPLIED

Spectrophotometer or filter photometer (370, 410 and 450 nm)

Centrifuge, thermostatic bath.

## PREPARATION OF WORKING REAGENTS

### REAGENT 4 (4/A + 4/B)

Add the contents of a vial of Reagent 4/A into a vial of Reagent 4/B.

Shake gently until complete dissolution.

STABILITY: at least 4 months at 2-8°C.

### REAGENT 5 standard

Dilute 1 volume of Reagent 5 standard with 5 volumes of absolute ethanol, before using in the test.

STABILITY: at least 2 months at 2-8°C.

## ELUENT

Dilute 7 volumes of absolute ethanol (not included in the kit) with 3 volumes of distilled water. For 40 assays (plus 8 standard and 8 blank reagent), 272 ml of eluent are required. It is suggested to prepare 280 ml of eluent diluting 196 ml of absolute ethanol with 84 ml of distilled water. Store in a tightly closed plastic bottle.

## SAMPLE

24-hour urine.

Collect the 24-hour urine in a container with 3-4 ml of 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: 370, 410, 450 nm  
Optical path: 1 cm  
Reading: against blank  
Temperature: 60 or 37°C  
Method: colorimetric endpoint  
Linearity: up to 50 mg/l  
Sensitivity: 0.6 mg/l  
Recovery: 92 ± 2 %  
C.V.: 2 %

## PREPARATION OF THE SAMPLE

Pipette the following into a centrifuge test-tube:

Reagent 6	1 level measure
Urine	5.0 ml
Reagent 1	drop by drop to have a pH between 2 and 3

Shake for at least 3 minutes and centrifuge at 4000 rpm for 5 minutes. Pour all the liquid into a centrifuge tube and add mixing:

Reagent 2	drop by drop to have a pH between 7 and 8
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Caused by the formation of aluminum hydroxide at neutral pH, a persistent opalescence will appear; do not confuse it with the eventual turbidity generated in the previous decantation operation.

Shake for 10 seconds and centrifuge at 4000 rpm for 5 minutes.

## PREPARATION OF THE COLUMN

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

## CHROMATOGRAPHIC SEPARATION

Pour all the supernatant centrifuged liquid in a column and let it completely drain. Pipette:

Distilled water	10.0 ml	discard the eluate
Eluent	6.0 ml	collect the eluate

Mix the eluate thoroughly.

STABILITY: at least 3 days at 2-8°C.

## COLORIMETRIC REACTION

Pipette the following into test-tubes with cap:

	Sample	Sample Blank	Standard	Standard Blank
Eluate	2.0 ml	2.0 ml	---	---
Reagent 5 (diluted standard)	---	---	0.1 ml	---
Eluent	---	---	1.9 ml	2.0 ml

Put the test-tubes into an ice-bath and pipette as it follows:

Reagent 3	---	2.5 ml	---	---
Reagent 4	2.5 ml	---	2.5 ml	2.5 ml

Mix repeatedly keeping the test-tubes into an ice-bath to avoid overheating. Incubate in a water bath for 15 minutes at 60°C or for 90 minutes at 37°C. Cool the tubes under running water, at room temperature. Read the sample (As) and the standard (Astd) absorbencies at 370, 410 and 450 nm against the blank sample and the blank standard, respectively.

## CALCULATION

Apply Allen correction formula to the sample and standard readings:

$$\Delta A = 2 A_{410} - (A_{370} + A_{450})$$

$$17\text{-OH CS (mg/L)} = (\Delta A_s / \Delta A_{std}) \times 10$$

$$17\text{-OH CS (mg/24 hours)} = \text{mg } 17\text{-OH CS} / L \times L \text{ of 24 hour urine}$$

## REFERENCE VALUES

Adult women 2 – 8 mg/24 hours

Adult men 3 – 12 mg/24 hours

## NOTES

1. Reagent quantities are enough for 56 tests (40 samples including its blank, 8 standards and 8 blanks).
2. 17-OHCS levels at sensibility limit may give negative  $\Delta A$  values. In this case, pipette double sample (10 ml of urine instead of 5 ml) and divide the result by 2.
3. Reaction volumes can be proportionally changed.

## REFERENCE

1. Y. Ariyoshi et Y. Osawa, Clin. Chem., 22, 232 (1976)



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Manufactured by: FAR srl

Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY

Phone +39 045 6700870 - Fax +39 045 7157763

website: <http://www.farddiag.com> e-mail: [farddiag@farddiag.com](mailto:farddiag@farddiag.com)