



DHEA, TESTOSTERONE, AND EPITESTOSTERONE IN URINE FOR GC OR GC/MS ANALYSIS USING:

200 mg CLEAN THRU[®] EXTRACTION COLUMN

Part #: ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU[®] Tips

1. PREPARE SAMPLE

Pipette 5 mL of urine into borosilicate glass test tubes.

Add internal standard*, adjust sample pH to 5.5 - 6.5 using concentrated sodium phosphate monobasic or dibasic.

Mix sample.

Centrifuge samples at 3000 rpm for 5 min.

2. CONDITION CLEAN SCREEN[®] EXTRACTION COLUMN

1 x 3 mL CH₃OH.

1 x 3 mL D.I. H₂O.

1 x 1 mL 100 mM phosphate buffer (pH 6.0).

3. APPLY SAMPLE

Pour supernatant onto column. Allow to flow via gravity.

4. WASH COLUMN

1 x 3 mL D.I. H₂O.

Dry column (10 minutes at > 10 mm Hg).

5. ELUTE STEROIDS

1 x 3 mL of CH₃OH.

Collect at 1 -2 mL/ minute.

6. ENZYMATIC HYDROLYSIS

Dry eluate under a stream of nitrogen; Add 2 mL of 200 mM phosphate buffer (pH 7.0) and 250 units of β glucuronidase Mix Vortex and allow to incubate at 50°C for 1 hour. Cool sample, cap and adjust the pH to 10-11 using a 1:1 mixture of NaHCO₃/Na₂CO₃.

7. ADDITIONAL CLEAN-UP[®]

Add 5 mL of n-butyl chloride to each sample. The tubes and shake vigorously for 10 minutes and then centrifuge at 3000 rpm for 5 min. Transfer the organic layer to clean test tubes and dry under a stream of nitrogen. Place dried sample in a desiccator and further dry under vacuum for 30 minutes.

8. DERIVATIZE

Add 50 μ L of MSTFA**/NH₄/dithioerythritol. (1000:2:5, V/W/W) and incubate at 70°C for 20 min.

Centrifuge sample at 3000 rpm for 1 min. and transfer directly to GC injector vials.

9. QUANTITATE

Inject 1 to 2 μ L onto gas chromatograph.

For MSD monitor the following ions:

<u>Compound</u>	<u>Primary Ion***</u>	<u>Secondary</u>
Testosterone	432	417
Epitestosterone	432	417
DHEA	432	417
16 α Hydroxytestosterone*	520	259

* Suggested internal standard at 20 ng/mL

** Part # SMSTFA-0-1, 10, 25, 100

*** Quantitation ion

SOURCE - UCT Internal Publication