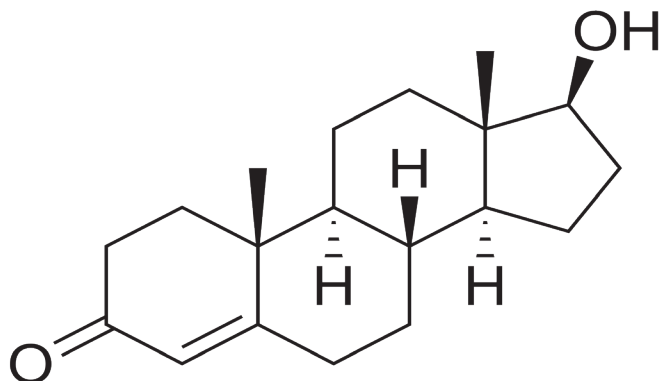


DHEA, Testosterone, and Epitestosterone In Urine For GC Or GC/MS Analysis Using: 200 mg Clean Thru® Extraction Column



UCT Part Numbers

ZSDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
Without Tips

Or

ZCDAU020
Clean Screen® DAU
10 mL, 200 mg sorbent
With CLEAN-THRU® Tips

Procedure:

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 µL of MSTFA**/NH₄I/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 µL onto gas chromatograph.
- For MSD monitor the following ions:

Analyte	Primary Ion***	Secondary
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



UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com
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