



**FREE (UNBOUND) OPIATES IN BLOOD, PLASMA/SERUM,
TISSUE FOR GC OR GC/MS CONFIRMATIONS**
USING: 200 mg CLEAN SCREEN[®] EXTRACTION COLUMN
Part # ZSDAU020 without Tips or ZCDAU020 with CLEAN-THRU[®] Tips

1. PREPARE SAMPLE

To 1 mL of 100 mM phosphate buffer (pH=6) add internal standards*. Add 1 mL of blood, plasma/ serum, or 1 g (1:4) tissue homogenate.

Mix/vortex and let stand 5 minutes

Add 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex

Sample pH should be 6.0 ± 0.5 .

Adjust pH accordingly with 100 mM monobasic or dibasic sodium phosphate.

Centrifuge for 10 minutes at 2000 rpm and discard pellet

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).

NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE

Load at 1 mL/minute.

4. WASH COLUMN

1 x 2 mL D.I. H₂O.

1 x 2 mL 100 mM acetate buffer (pH 4.5).

1 x 3 mL CH₃OH.

Dry column (5 minutes at >10 inches Hg).

5. ELUTE OPIATES

1 x 3 mL CH₂Cl₂/IPA/NH₄OH (78:20:2);

Collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

Add IPA/NH₄OH, mix, then add CH₂Cl₂ (pH 11-12).

6. DRY ELUATE

Evaporate to dryness at < 40°C.

7. DERIVATIZE

Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS)^{***}.

Overlay with N₂ and cap. Mix/vortex.

React 30 minutes at 70°C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

8. 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>	<u>Tertiary</u>	<u>Cerilliant#</u>
Codeine- D3-TMS*	374	237	346	C-039
Codeine-TMS:	371	234	343	C-015
Morphine-D3-TMS*	432	290	327	M-003
Morphine-TMS	429	287	324	M-005

* Suggested internal standard for GC/MS: Codeine-D3, Morphine-D3

*** Part # SBSTFA-1-1,10, 25, 100

**** Quantitation ion