1. PREPARE SAMPLE
   To 2 mL urine add internal standard(s)* and 1 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.

2. CONDITION 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 to 2 mL/minute.

4. WASH COLUMN
   1 x 3 mL D.I. H₂O.
   1 x 2 mL 100 mM HCl.
   1 x 3 mL CH₃OH.
   Dry column (5 minutes at > 10 inches Hg).

5. ELUTE COCAINE AND BENZOYLECGONINE
   1 x 3 mL Methylene Chloride/Isopropanol/
   Ammonium Hydroxide (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 20 minutes at 70°C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

   **Quantitation Ion**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Primary Ion****</th>
<th>Secondary</th>
<th>Tertiary</th>
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<tbody>
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<td>303</td>
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<tr>
<td>D₃-Benzoylcoine-TMS*</td>
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<td>364</td>
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<td>Benzoylecgonine-TMS</td>
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<td>256</td>
<td>361</td>
</tr>
</tbody>
</table>

* Suggested internal standards for GC/MS: D₃-Cocaine, D₃-Benzoylcoine

** Quantitation Ion

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COCaine AND BENZOYLECGONINE IN UrINE FOR GC OR GC/MS CONFIRMATIONS

Part # 60108-742

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COCaine AND BENZOYLECGONINE IN SERUM, PLASMA, OR WHOLE BLOOD FOR GC OR GC/MS CONFIRMATIONS

Part # 60108-742

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1. PREPARE SAMPLE
   To 1 mL of sample (Serum, Plasma or Whole Blood) add internal standard* and 4 mL of D.I. H₂O.
   Mix/vortex and let stand 5 minutes.
   Centrifuge for 10 minutes at 2,000 rpm and discard pellet.
   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.

2. CONDITION 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 to 2 mL/minute.

4. WASH COLUMN
   1 x 3 mL D.I. H₂O.
   1 x 2 mL 100 mM HCl.
   1 x 3 mL CH₃OH.
   Dry column (5 minutes at > 10 inches Hg).

5. ELUTE COCAINE AND BENZOYLECGONINE
   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 20 minutes at 70°C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

   **Quantitation Ion**

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</tbody>
</table>

* Suggested internal standards for GC/MS: D₃-Cocaine, D₃-Benzoylcoine

** Quantitation Ion

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THE HOUSE OF CHROMATOGRAPHY

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COCAINE AND BENZOYLECGONINE IN SERUM, PLASMA, OR WHOLE BLOOD FOR HPLC

Part # 60108-742

1. PREPARE SAMPLE
   To 1 mL of sample (Serum, Plasma or Whole Blood) add internal standard(s) and 4 mL of D.I. H2O.
   Whole Blood: Mix/vortex and let stand 5 minutes.
   Centrifuge for 10 minutes at 2000 rpm and discard pellet.
   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.

2. CONDITION 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 to 2 mL/minute.

4. WASH COLUMN
   1 x 3 mL D.I. H2O.
   1 x 2 mL 100 mM HCl.
   1 x 3 mL CH3OH.
   Dry column (5 minutes at > 10 inches Hg).

5A*. ELUTE COCAINE AND BENZOYLECGONINE
   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).

5B*. ELUTE COCAINE AND BENZOYLECGONINE
   1 x 2 mL CH3OH/NH4OH (98:2); collect eluate at 1 to 2 mL/minute.
   NOTE: Prepare elution solvent daily.
   Add 3 mL D.I. H2O and 500 µL CH2Cl2 to eluate.
   Mix / vortex 10 seconds. Centrifuge if necessary to separate layers.
   Aspirate and discard aqueous (upper) layer.

6. CONCENTRATE
   Evaporate to dryness at < 40°C.
   Reconstitute in mobile phase for injection into HPLC.

* Choose either 5A or 5B

COCAINE AND ITS METABOLITES FROM MECONIUM FOR GC OR GC/MS ANALYSIS

Part # 60108-742

1. PREPARE SAMPLE
   Vortex 0.5 -1 g meconium and 2 mL of CH3OH.
   Centrifuge and transfer the supernatant to a clean tube.
   To each tube add 3 mL 100 mM phosphate buffer (pH 6.0), internal standard and vortex.
   Matrix must be more aqueous than organic for good extraction to occur.

2. CONDITION EXTRACTION COLUMN
   1 x 3 mL CH3OH.
   1 x 3 mL D.I. H2O.
   1 x 3 mL 100 mM phosphate buffer (pH 6.0).
   NOTE: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. APPLY SAMPLE
   Load at 1 to 2 mL/minute. Allow to dry.

4. WASH COLUMN
   1 x 3 mL D.I. H2O.
   1 x 1 mL 100 mM HCl.
   1 x 3 mL CH3OH.
   Dry column (5 minutes at > 10 inches Hg).

5. ELUTE COCAINE AND METABOLITES
   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. EVAPORATE
   Evaporate the elution solvent to dryness without heating.

7. DERIVATIZE
   Add 50 µL ethyl acetate and 50 µL BSTFA (with 1% TMCS).
   Overlay with N2 and cap. Mix/vortex.
   React 20 minutes at 70°C. Remove from heat source to cool.
   NOTE: Do not evaporate BSTFA solution.

8. QUANTITATE
   Inject 1 to 2 µL onto gas chromatograph.
   For MSD monitor the following ions:

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**** Quantitation ion

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