A Rapid iMethod™ Test for Estrogens Analysis

**iMethod™ Test for Estrogens V.1.0 for Cliquid® Software**

The following information outlines the instrument requirements and expected results from the AB SCIEX iMethod™ Test for the quantitation of Estrogens in human serum when using an AB SCIEX API 5000™ or Triple Quad 5500™ LC/MS/MS System. This iMethod™ Test contains a method for Estrone and Estradiol and a second method for Estriol due to the use of different extraction protocols.

Sample preparation is based upon a liquid-liquid extraction using a high purity hexane/ethyl acetate mixture followed by injection on a Phenomenex Phenyl C6 3 um HPLC column, included with the method, connected to the Turbo V™ ion source of the mass spectrometer. Both tests have an LC run time of 6.0 minutes per sample.

![Example chromatogram for 15 pg/ml of Estrone and Estradiol generated on an API 5000™ system with a Shimadzu Prominence LC system in a six-minute run. D4-Estrone and D3-Estradiol are used as internal standards](image)

During the method evaluation for Estrone (E1) and Estradiol (E2), quantitation limits for each analyte were sufficient to allow the analytical method to be used for quantitation with a linear range from 5 to 500 pg/mL. A typical intra-assay precision (n=3) for the low QC of 15 pg/mL concentration for Estrone gives a %CV value of 10 with an accuracy of 90% and a signal-to-noise (S/N) of 55. A typical intra-assay precision (n=3) for the low QC of 15 pg/mL concentration for Estradiol gives a %CV value of 10 with an accuracy of 90% and a signal-to-noise (S/N) of 43. In both cases, S/N is the peak height divided by the noise measured at 3 standard deviations of the noise.
Figure 2. Example chromatogram for 30 pg/ml of Estriol generated on an API 5000™ system with a Shimadzu Prominence LC system in a six-minute run. D3-Estriol was used as the internal standard.

During the method evaluation for Estriol, the quantitation limits were sufficient to allow the analytical method to be used for quantitation over a linear range of 10 to 500 pg/mL. Typical intra-assay precision (n=3) for the low QC concentration of 30 pg/mL is a 10 %CV and an accuracy of 90% with a signal-to-noise (S/N) of 40. The S/N is the peak height divided by the noise measured at 3 standard deviations of the noise.

Calibration

The following calibration curves for Estrone and Estradiol represent the linear dynamic range of from 5-500 pg/ml, as expected for this assay. The following calibration curve for Estriol shows a linear dynamic range of 10-500 pg/ml and is representative of the data expected for this assay.

Figure 3. Representative calibration curve for Estrone. Experiment was performed on an API 5000™ LC/MS/MS system with a Shimadzu Prominence LC system in a six-minute run. The assay was linear across the concentration range of 5-500 pg/ml.
Figure 4. Representative calibration curve for Estradiol. Experiment was performed on an API 5000™ LC/MS/MS system with a Shimadzu Prominence LC system in a six-minute run. The assay was linear across the concentration range of 5-500 pg/ml.

Figure 5. Representative calibration curve for Estriol. Experiment was performed on an API 5000 LC/MS/MS system with a Shimadzu Prominence LC system in a six-minute run. The assay was linear across the concentration range from 10-500 pg/ml.

Please note that the Phenomenex Phenyl C6 HPLC column is included with this iMethod™ Test. Also, that this method can also be run on other HPLC systems, given that they are supported for use by Cliiquid® Software and the retention times are updated to reflect the configuration used.
System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX API 5000™ or AB SCIEX Triple Quad™ 5500 LC/MS/MS System
- A Shimatzu Prominence 20A HPLC system with reservoir tray and bottles, CBM-20A system controller, 100 µl mixer, 2 isocratic LC-20AD pumps, 3 channel degasser, SIL-20AC autosampler and column oven or an Agilent 1100/1200 HPLC system with binary pump (no static mixer), well plate autosampler and thermostated column oven.
- Phenomenex Phenyl C6 3 um 50 x 2 mm HPLC column
- Charcoal filtered serum and defibrinated double charcoal treated serum (www.goldenwestbio.com)
- HPLC grade Hexane and Ethyl Acetate (www.sigmaaldrich.com)
- HPLC grade water, methanol and ammonium hydroxide (www.sigmaaldrich.com)
- Borosilicate tubes
- Plain centrifuge tubes
- A vortex mixer
- Pipettes and standard laboratory glassware

Ordering Information

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Part Number</th>
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<tr>
<td>iMethod™ Test for Estrogens V.1.0 for Cliquid® Software</td>
<td>1038488</td>
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While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for Estrogens, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod™ Test and are recommended for inexperienced users. Please consult your local sales representative for more details.

Important Note

The purchase and use of certain chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements.

The iMethod™ Test described above has been developed by AB SCIEX to provide all the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. The performance of this method will need to be verified in a given lab due to potential variations in instrument performance, maintenance, chemicals and procedures used, technical experience, sample matrices and environmental conditions. It is the responsibility of the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

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