# Analysis of Urine Using the MxP® Quant 500 Kit with SCIEX 5500+ and 6500+

A protocol for the analysis of urine samples using the MxP® Quant 500 kit with SCIEX QTRAP 5500+ and 6500+ is described below. For other instruments, please inquire with the Biocrates customer support. The workflow is slightly different from the one described in the MxP® Quant 500 kit user manual and additional items are required, as listed in the table below.

In general, we recommend performing pilot tests with a representative sample set before starting a larger study. The results may vary depending on the nature and the quality of the samples. Due to the high salt content of urine samples, it is recommended to clean the instrument after each kit. Biocrates does not assume responsibility for the results or possible system contamination.

The following items are required in addition to the standard MxP® Quant 500 kit content:

| ***Kit Item*** | ***Description*** | ***Details*** |
|---|---|---|
| Met*IDQ*™ patch: *MStype*_Q500_Urine_ DB110_*YYMMDD*.jar (provided by customer support) | To be imported into the Met*IDQ*™ database | Loads the OPs for urine analysis into Met*IDQ*™ (required to generate the worklist) |
| Absolute*IDQ*® p180 Cal1 - Cal7, 7 vials | Calibration standards, lyophilized | Used instead of the regular MxP® Quant 500 calibrators |
| Absolute*IDQ*® p180 Crea Cal1 - Cal7, 7 vials | Calibration standards creatinine, lyophilized | Used as additional calibrators |
| Absolute*IDQ*® p150/p180 ISTD Urine Crea, 1 vial | Internal standard creatinine, lyophilized | Used as additional internal standard |
| Absolute*IDQ*® p150/p180 Urine Zero Sample, 1 vial | Solution of 350 mM urea, 15 mM $NaHPO_4$, pH 6.0 (urine imitation) | Used as zero sample instead of PBS |
| Recipe Urine QC, 1 vial | Lyophilized urine | Used as additional quality control |
| Acquisition Methods for SCIEX Analyst Software (provided by support) | MxP500L-LC1_5xxx01_**Urine**.dam MxP500L-LC2_5xxx.dam (regular) MxP500F-FIA1_5xxx01_**Urine**.dam | Used for data acquisition |

| Kit Item | Description | Details |
|---|---|---|
| | MxP500F-FIA2_5xxx.dam (regular) | (version number depends on MS type and HPLC or UHPLC mode) |

## 1. Met*IDQ*™ Software – Worklist Generation in MetLIMS

| Step | Instruction |
|---|---|
| 1 | Install the patch ***MSType_Q500_Urine_DB110_YYMMDD.jar*** (provided by customer support) according to Appendix "Installing Database Patches" of the Met*IDQ*™ user manual. |
| 2 | Select the material class "(40) urine", when you register urine samples. |
| 3 | Select the correct OP for your instrument, when you generate the LC worklist for urine: **MXP500L-40-5xxx01** |
| 4 | Worklists Settings > Zero Samples: unlink PBS and link Urine imitation (barcode 11000007). |
| 5 | Worklists Settings > Standards: link the following Calibration Standards: |
| 6 | Link QCs and experimental samples as usual. |
| 7 | Duplicate the LC plate (copy and paste derived plate) as usual and change the OP of the copied plate to the corresponding one for FIA: **MXP500F-40-5xxx01**<br>Delete the calibration standards in the "Plate View" of the FIA plate. |

| Sample Bar Code | Sample Type | Sample Identification |
|---|---|---|
| 1020511834 | Standard L0.25 | Quant 500 Urine Cal0.25 |
| 1020511849 | Standard L0.5 | Quant 500 Urine Cal0.5 |
| 1020511853 | Standard L1 | Quant 500 Urine Cal1 |
| 1020511868 | Standard L2 | Quant 500 Urine Cal2 |
| 1020511872 | Standard L3 | Quant 500 Urine Cal3 |
| 1020511887 | Standard L4 | Quant 500 Urine Cal4 |
| 1020511891 | Standard L5 | Quant 500 Urine Cal5 |
| 1020511902 | Standard L6 | Quant 500 Urine Cal6 |
| 1020510481 | Standard L7 | Quant 500 Urine Cal7 |
| 1020510495 | Standard L8 | Quant 500 Urine Cal8 |

## 2. Preparing the Kit in the Lab

<table>
<tr><th>Step</th><th>Instruction</th></tr>
<tr><td>1</td><td>Resuspend all vials using LC-MS grade water:<br>a) ISTD Urine Crea: add 1200 µL<br>b) AbsoluteIDQ® p180 Cal1 - Cal6: add 100 µL<br>c) AbsoluteIDQ® p180 Crea Cal1 - Cal6: add 100 µL<br>d) AbsoluteIDQ® p180 Cal7: add 50 µL<br>e) AbsoluteIDQ® p180 Crea Cal7: add 50 µL<br>f) MxP® Quant 500 QC1 - QC3: add 100 µL<br>g) Recipe Urine QC: add 1000 µL</td></tr>
<tr><td>2</td><td>Shake all vials for 15 min at 1200 rpm. Afterwards, vortex several times (before use).</td></tr>
<tr><td>3</td><td>Add 10 µL of the “ISTD Urine Crea” to each well of the kit plate except the blank well A1. Pipette directly onto the filters of the kit plate. Do not pipette on the inner wall of the wells or on the plastic holder. Use an Eppendorf Multipette (or other repeater pipette), adjust to maximum dispensing speed.</td></tr>
<tr><td>4</td><td>At this point, an extra drying step is required before you continue. Dry all wells for 15 min under nitrogen according to the manual.</td></tr>
<tr><td>5</td><td>Load and combine the resuspended calibration standards from step 1 directly on the kit plate as follows (according to your plate map):
<table>
<tr><th>Calibration level</th><th>Volume from vial “p180 Cal”</th><th>Volume from vial “p180 Crea Cal”</th></tr>
<tr><td>Cal 0.25</td><td>2.5 µL of p180 Cal 1</td><td>2.5 µL of p180 Crea Cal 1</td></tr>
<tr><td>Cal 0.5</td><td>5 µL of p180 Cal 1</td><td>5 µL of p180 Crea Cal 1</td></tr>
<tr><td>Cal 1</td><td>10 µL of p180 Cal 1</td><td>10 µL of p180 Crea Cal 1</td></tr>
<tr><td>Cal 2</td><td>10 µL of p180 Cal 2</td><td>10 µL of p180 Crea Cal 2</td></tr>
<tr><td>Cal 3</td><td>10 µL of p180 Cal 3</td><td>10 µL of p180 Crea Cal 3</td></tr>
<tr><td>Cal 4</td><td>10 µL of p180 Cal 4</td><td>10 µL of p180 Crea Cal 4</td></tr>
<tr><td>Cal 5</td><td>10 µL of p180 Cal 5</td><td>10 µL of p180 Crea Cal 5</td></tr>
<tr><td>Cal 6</td><td>10 µL of p180 Cal 6</td><td>10 µL of p180 Crea Cal 6</td></tr>
<tr><td>Cal 7</td><td>5 µL of p180 Cal 7</td><td>5 µL of p180 Crea Cal 7</td></tr>
<tr><td>Cal 8</td><td>10 µL of p180 Cal 7</td><td>10 µL of p180 Crea Cal 7</td></tr>
</table>
Use a single-channel pipette to pipette the volumes according to the table directly onto the center of each filter. Gently touch the filter inserts with the pipette tip while pipetting the samples. Do not pipette on the inner wall of the wells or on the plastic holder and avoid cross-contamination. Use a fresh tip for each sample.</td></tr>
<tr><td>6</td><td>Load 10 µL of all other samples (zero sample, QCs and experimental samples) as usual according to the manual and according to your plate map. Use a single-channel pipette to pipette 10 µL onto the center of each filter. Gently touch the filter inserts with the pipette tip while</td></tr>
</table>

| | |
|---|---|
| | pipetting the samples. Do not pipette on the inner wall of the wells or on the plastic holder and avoid cross-contamination. Use a fresh tip for each sample. |
| **7** | Dry all wells for 30 min under nitrogen according to the manual. |
| **8** | Derivatization:<br>The derivatization solution is different from the regular manual. Remove the phenyl isothiocyanate (PITC) from the freezer and allow to equilibrate to room temperature. In the meanwhile, prepare the pre-mix and mix the following chemicals in the plastic tube that you find in the kit box:<br>a) 4.2 mL of methanol (LC-MS grade)<br>b) 0.6 mL of water (LC-MS grade)<br>c) 0.6 mL of triethylamine (≥99% purity)<br>Vortex for 10 sec. |
| **9** | Prepare the derivatization solution and add 0.6 mL of PITC to the pre-mix. Vortex for 10 sec. |
| **10** | Add 50 µL to each well. The derivatization time at room temperature is 20 min. |
| **11** | Continue with the regular manual and dry all wells for 60 min under nitrogen. |

## 3. Instrumental Setup

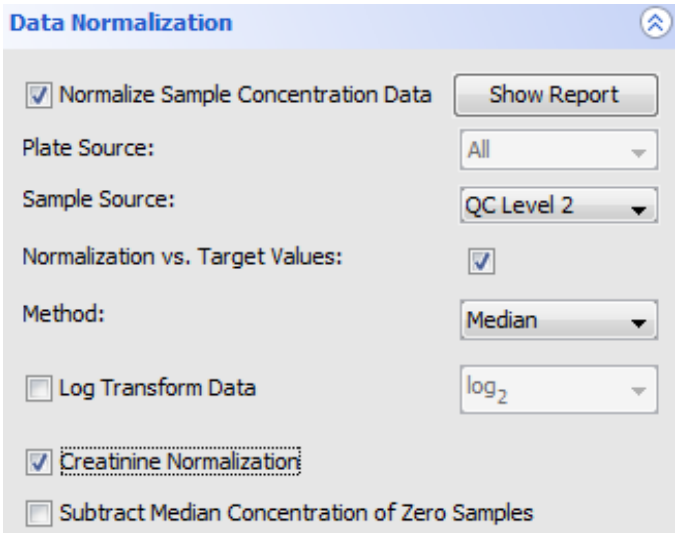
| ***Step*** | ***Instruction*** |
|---|---|
| **1** | Copy the Analyst acquisition methods into the corresponding folder of your Analyst project. |
| **2** | Setup the acquisition methods according to section 8 of the MxP® Quant 500 kit user manual. |

## 4. Met*IDQ*™ Software – Creatinine Normalization and Data Export in MetSTAT

Creatinine is used for normalization (automatically performed in Met*IDQ*™). Please refer to Waikar et al., Kidney Int 2010; 78(5):486-94.

| ***Step*** | ***Instruction*** |
|---|---|
| | Since creatinine is analyzed and quantified in both parts separately, LC and FIA, the results must be normalized and exported one after the other and must not be merged. |
| **1** | Go to **MetSTAT > Select Samples** and link the samples of the LC run only. |
| **2** | Go to the **Display Data** tab and find the tool **Data Normalization** on the right sidebar (see screenshot below). |
| **3** | Activate the checkbox **Creatinine Normalization** and all metabolite concentrations will be automatically divided by the creatinine concentration in each sample. All other Met*IDQ*™ functions can be used as usual, such as QC normalization. |

| Step | Instruction |
|---|---|
| 4 | Export the results table and repeat the steps 1-3 for the FIA run. |




## 5. Analytical Specifications for Creatinine

| Analyte | LOD (µM) | LLOQ (µM) | ULOQ (µM) |
|---|---|---|---|
| Creatinine | 10 | 500 | 30,000 |

You can reach us via e-mail (support@biocrates.com) or telephone:

Stephen Dearth, PhD: +1 704 4216512 (North America)
Dr. Manuel Kratzke: +43 676 848434 105 (Rest of World)
Dr. Markus Langsdorf: +43 676 848434 214 (Rest of World)