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The future of research and health

Analysis of urine samples using the MxP® Quant 500 kit

Content

1 Introduction and additional items

A protocol for the analysis of urine samples using the MxP® Quant 500 kit is described below. The workflow is slightly different from the one described in the 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 every 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 contents

Additional items	Description	Details
Triethylamine Purchased by customer	Purity ≥99%	Used for derivatization solution instead of pyridine
MetIDQ™ patch: MStype_Q500_urine_DB110_YYMMDD.jar (provided by support)	Patch containing urine analysis protocols for MetIDQ™	To be imported into MetIDQ™ in addition to the regular Quant 500 patch, required to generate the worklist
AbsoluteIDQ® p180 Cal1 – Cal7, 7 vials	Calibration standards, lyophilized	Used instead of the regular Quant 500 calibration standards
AbsoluteIDQ® p150/p180 ISTD Urine Crea, 1 vial	Internal standard creatinine, lyophilized	Additional creatinine internal standard to be added to plate
AbsoluteIDQ® 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 control, 1 vial	Lyophilized urine QC sample	Used as additional quality control
Methods for Agilent MassHunter software (provided by support)	**LC1:** MxP500L-LC1_7x11_urine.m **FIA1:** MxP500F-FIA1_7x11_urine.m **LC2, FIA2 and FIA3:** regular according to manual	Used for data acquisition. **The specific numbers (x) depend on MS type, see Quant 500 and MetIDQ™ user manuals.**
Methods for Waters MassLynx® software (provided by support)	**LC1:** MxP500L-LC1_8xx2_urine.exp **FIA1:** MxP500F-FIA1_8xx2_urine.exp **LC2 and FIA2 (and FIA3 for TQ-S):** regular according to manual	Used for data acquisition. **The specific numbers (xx) depend on MS type, see Quant 500 and MetIDQ™ user manuals.**
Methods for SCIEX Analyst® software (provided by support)	**LC1:** MxP500L-LC1_5xx2_urine.dam **FIA1:** MxP500F-FIA1_5xx2_urine.dam **LC2 and FIA2:** regular according to manual	Used for data acquisition. **The specific numbers (xx) depend on MS type, see Quant 500 and MetIDQ™ user manuals.**

2 MetIDQ™ software – Generate worklist in MetLIMS

<table>
<tr><th>Step</th><th>Instruction</th></tr>
<tr><td>1</td><td>Install the patch MStype_Q500_urine_DB110_YYMMDD.jar (provided by support) according to MetIDQ™ user manual section “Installing database patches”.</td></tr>
<tr><td>2</td><td>Select the material class “(40) urine”, when you register urine samples.</td></tr>
<tr><td>3</td><td>Select the correct OP for your instrument when you generate the LC worklist for urine: MXP500L-40-xxxx</td></tr>
<tr><td>4</td><td>In the Zero Samples tab of the Worklists Settings For Submission window, unlink PBS and link Urine imitation (barcode 11000007).</td></tr>
<tr><td>5</td><td>In the Standards tab of the Worklists Settings For Submission window, link the following calibration standards:
<table>
<tr><th>Sample Bar Code</th><th>Sample Type</th><th>Sample Identification</th></tr>
<tr><td>1020509951</td><td>Standard L0.25</td><td>p180 Cal0.25</td></tr>
<tr><td>1020509966</td><td>Standard L0.5</td><td>p180 Cal0.5</td></tr>
<tr><td>20000611</td><td>Standard L1</td><td>p180 Cal1</td></tr>
<tr><td>20000612</td><td>Standard L2</td><td>p180 Cal2</td></tr>
<tr><td>20000613</td><td>Standard L3</td><td>p180 Cal3</td></tr>
<tr><td>20000614</td><td>Standard L4</td><td>p180 Cal4</td></tr>
<tr><td>20000615</td><td>Standard L5</td><td>p180 Cal5</td></tr>
<tr><td>20000616</td><td>Standard L6</td><td>p180 Cal6</td></tr>
<tr><td>20000617</td><td>Standard L7</td><td>p180 Cal7</td></tr>
<tr><td>1020509971</td><td>Standard L8</td><td>p180 Cal8</td></tr>
</table></td></tr>
<tr><td>6</td><td>In the QC-Lots tab of the Worklists Settings For Submission window, link the regular Quant 500 QC levels 1-3, as well as the Recipe Urine QC (QC Level 0). Adjust at least 3 replicates of both, Quant 500 QC2 and Recipe Urine QC, and distribute over the plate.</td></tr>
<tr><td>7</td><td>In the Samples tab of the Worklists Settings For Submission window, link the experimental samples as usual.</td></tr>
<tr><td>8</td><td>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-xxxx</td></tr>
<tr><td>9</td><td>According to the regular Quant 500 workflow (see user manual), delete the calibration standards in the Plate View of the FIA plate, set the Condition to Approved and perform Export Worklist for MS.</td></tr>
</table>

3 Instrumental setup

Step	Instruction
1	On the MS operating computer, copy the instrument-specific acquisition methods (see page 1 of this document) into the corresponding folder of your biocrates project.
2	Install the acquisition methods according to section 4 of the MxP® Quant 500 kit user manual.
3	Continue with the MxP® Quant 500 kit user manual and run the SST and the kit.
	Do not thaw samples or start with the kit before successful completion of the SST.

4 Preparing the kit in the lab

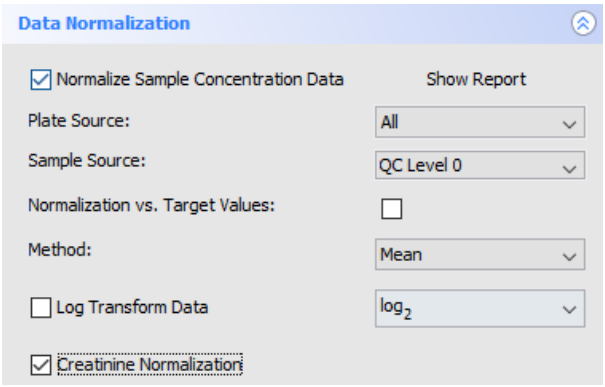
Step	Instruction
1	Resuspend all vials using LC-MS grade water: Item / Volume ISTD Urine Crea — add 1200 µL AbsoluteIDQ® p180 Cal1 - Cal6 — add 100 µL AbsoluteIDQ® p180 Cal7 — add 50 µL MxP® Quant 500 QC1 - QC3 — add 100 µL Recipe Urine QC — add 1000 µL
2	Shake all vials for 15 min at 1200 rpm and vortex several times before use.
3	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 a repeater, e.g. Eppendorf Multipette®, adjusted to maximum dispensing speed.

<table>
<tr><th>Step</th><th>Instruction</th></tr>
<tr><td>4</td><td>Load 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></tr>
<tr><td>Cal 0.25</td><td>2.5 µL of p180 Cal 1</td></tr>
<tr><td>Cal 0.5</td><td>5 µL of p180 Cal 1</td></tr>
<tr><td>Cal 1</td><td>10 µL of p180 Cal 1</td></tr>
<tr><td>Cal 2</td><td>10 µL of p180 Cal 2</td></tr>
<tr><td>Cal 3</td><td>10 µL of p180 Cal 3</td></tr>
<tr><td>Cal 4</td><td>10 µL of p180 Cal 4</td></tr>
<tr><td>Cal 5</td><td>10 µL of p180 Cal 5</td></tr>
<tr><td>Cal 6</td><td>10 µL of p180 Cal 6</td></tr>
<tr><td>Cal 7</td><td>5 µL of p180 Cal 7</td></tr>
<tr><td>Cal 8</td><td>10 µL of p180 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>5</td><td>Load 10 µL of all other samples (zero sample, QCs and study samples) as usual according to the user manual and 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 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>Dry all wells for 30 min under nitrogen according to the user manual.</td></tr>
<tr><td>7</td><td>Derivatization (different from the regular manual):
Prepare the derivatization pre-mix by adding the following chemicals to the plastic tube included in the kit box:
– 4.2 mL of methanol (LC-MS grade)
– 0.6 mL of water (LC-MS grade)
– 0.6 mL of triethylamine (≥99% purity)
Vortex for 10 sec.</td></tr>
<tr><td>8</td><td>Remove the phenyl isothiocyanate (PITC) from the freezer and allow to equilibrate to room temperature. Prepare the derivatization solution by adding 0.6 mL of PITC to the derivatization pre-mix. Vortex for 10 sec.</td></tr>
<tr><td>9</td><td>Add 50 µL of the derivatization solution to each well using a repeater, e.g. Eppendorf Multipette®. The derivatization time at room temperature is 20 min.</td></tr>
<tr><td>10</td><td>Continue with the standard manual protocol (Step 7 – Quant 500 manual) and dry all wells for 60 min under nitrogen.</td></tr>
</table>

5 MetIDQ™ software – Normalization and data export

Data normalization can be performed using the **Recipe Urine QC**. Furthermore, creatinine is used specifically for normalization of urine concentrations (please refer to Waikar et al., Kidney Int 2010; 78(5):486-94). Both normalizations can be automatically performed in MetIDQ™.

Step	Instruction
1	Go to **MetSTAT > Select Samples** and link the samples of all plate runs of your urine project. Make sure there are two lines for each linked sample with plate barcode and OP belonging to the LC and FIA injection, respectively (except calibration standards which are only measured in the LC part). This will merge the LC and FIA data what is **required** for the creatinine normalization (step 3).
2	Go to the **Display Data** tab and use the normalization settings in the tool **Data Normalization** on the right sidebar as shown in the screenshot below. In this case, QC Level 0 is the **Recipe Urine QC**. Important: Do not normalize using QC Level 2, which is the regular Quant 500 QC2 (plasma-based), as this will significantly diminish the urine results.
3	Activate the checkbox **Creatinine Normalization** and each metabolite concentration will be automatically divided by the creatinine concentration.



6 Analytical specifications for creatinine

Analyte	LOD (µM)	LLOQ (µM)	ULOQ (µM)
Creatinine	100	500	30,000

Please check our support FAQ for solutions to common performance issues and technical problems or contact us: support@biocrates.com.