TECHNICAL NOTE

Codeplex Urine Sample Analysis: Workflow for Normalization to Creatinine

The following workflow has been used for the normalization of highly multiplexed CodePlex Secretome urine sample data to creatinine

In this Technical Note we outline:

- Urine proteomic biomarker analysis with CodePlex Secretome
- Obtaining and exporting analyte concentration data from IsoSpeak
- Normalizing analyte concentration data to creatinine concentration of sample

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Prep, Run, Analyze

Urine sample workflow using Bruker's highly multiplexed CodePlex solution

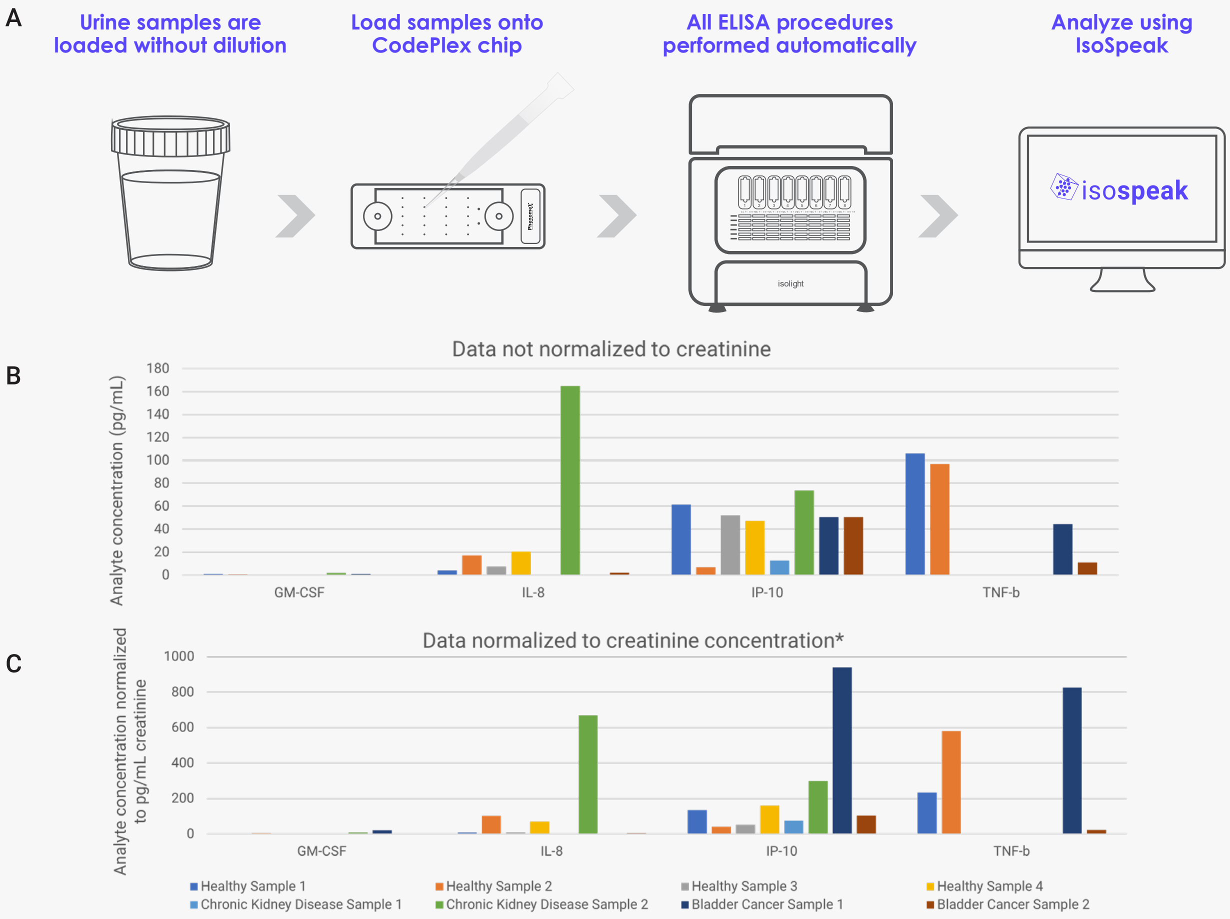

Figure 1 | A) Collected urine samples are loaded on the CodePlex Secretome chip. The Bruker system automates the entire ELISA workflow. B-C) Certain data trends are strengthened (IL-8 signal intensity), and other trends are revealed (IP-10, TNF-b signal intensity) when we normalize to the creatinine concentration of each sample.

Urine Proteomic Biomarker Analysis with CodePlex Secretome

As a direct filtrate of the blood, urine represents a non-invasive and easily accessible patient sample that can be screened for biomarkers indicative of disease. Urine biomarkers have been validated for renal and urogenital diseases such as acute kidney injury, bladder cancer, and diabetic nephropathy. Additionally, because urine can contain filtered plasma proteins, it has also been utilized in the assessment of proteomic biomarkers for non-renal diseases, including cardiovascular, autoimmune, pre-eclampsia, infectious diseases, and cancer.

In contrast to traditional ELISA assays, Bruker's CodePlex technology for highly multiplexed bulk proteomics enables an efficient and streamlined workflow. Low volumes of urine (11 uL per replicate) can be loaded directly onto CodePlex chips without dilution, where 30+ cytokines can be measured in a fully automated, end-to-end analysis (Figure 1A).

*Creatinine testing via Creatinine Parameter Assay, R&D Systems

Prep, Run, Analyze

Samples	Creatinine concentration (pg/mL) Data from creatine concentration determination kit (*not supplied by IsoPlexis*)	Creatinine normalization factor	Analyte 1 concentration (pg/mL) Data exported from IsoSpeak	Analyte 1 normalized	Analyte 2 concentration (pg/mL) Data exported from IsoSpeak	Analyte 2 normalized
Sample 1	**1.01 x 10^9**	1.01 x 10^9 /2.40 x 10^9 = **0.42**	**179.17**	179.17/0.42 = **426.595**	**96.86**	96.86/0.42 = **230.61**
Sample 2	**4.06 x 10^8**	4.06 x 10^8 /2.40 x 10^9 = **0.17**	**146.01**	146.01/0.17 = **858.88**	**60.26**	60.26/0.17 = **354.47**
Sample 3	**2.40 x 10^9**	2.40 x 10^9 /2.40 x 10^9 = **1.0**	**332.90**	332.90/1.0 = **332.90**	**45.12**	45.12/1.0 = **45.12**
Sample 4	**6.93 x 10^8**	6.93 x 10^8 /2.40 x 10^9 = **0.29**	**128.71**	128.71/0.29 = **443.82**	**129.55**	129.55/0.29 = **446.72**

Table 1: Creatinine normalization using a sample data set. Sample dataset for using creatinine concentration to create a normalization factor and applying the normalization factor to each analyte analyzed on CodePlex.

In healthy individuals, creatinine is produced and excreted at a constant rate. The uniformity of creatinine excretion facilitates its use as a standard for normalizing other protein levels in urine samples.

Obtaining and exporting analyte concentration data from IsoSpeak

1. Analyze your data via IsoSpeak.
2. To obtain concentration information for each analyte on the CodePlex chip panel, go to **"Data Overview"** tab found along the top banner in IsoSpeak. Under **"Select table type"**, select **"Signal Intensity"**.
3. Under the **"Table Options"** tab on the left, unselect **"Log Transform Signal Data"**.
4. While under **"Table Options"** tab, you can choose how you'd like to sort and label the data using the **"Label Sample By…"** and "**Sort Sample By…"** drop-down choices.
5. Under the **"Filter Selection"** tab on the left, select **"Quantify data"** to obtain pg/mL concentration data for each analyte. (Optional: select **"Avg. Replicates"** to obtain data for averaged replicates.)
6. There should be a populated table with the analytes from the CodePlex panel across the top of the table. The table is organized by each sample, noted in the first column of the table. To export the data table, select the **"Export"** button in the upper right.
7. Within the **"Export Options"** window, under **"Views"**, select **"Signal Intensity"**. Under **"Options"**, you can change the prefix of the exported file. Leave **"Export type"** as CSV. Select **"OK"** button in the lower right corner of the **"Export Options"** window and then select a preferred save location.

Normalizing analyte concentration data to creatinine concentration of sample

1. Determine creatinine concentration of your samples via assay that compares sample creatinine to a known creatinine standard *(assay not supplied by Bruker)*.
2. Normalize the creatinine concentration for each of your samples by the highest relative creatinine concentration (e.g., Sample 3, see "Creatinine normalization factor" column in table below).
3. Divide the concentration of analyte (exported from IsoSpeak) by the creatinine normalization factor to obtain normalized CodePlex Urine data.