

Centre for Organismal Studies
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## **ANALYSIS OF THIOLS**

**Description:** UPLC-Fluorescence (FLR) method for the sensitive detection and quantification of thiols. Detection limits depend on matrix type and input quantity. Samples are extracted using 0.1M HCl, semi-purified, derivatized using Monobromobimane and measured by UPLC-FLR.

Upon request reduced and oxidized Glutathione (GSH / GSSG) can be determined.

Analytes are reported as µM or pmol/mio cells or pmol/mg tissue.

**Container:** Eppendorf Tube or equivalent

**Optimal Volume:** Plasma / cell culture medium (100 μl); Tissue (25 mg)<sup>1</sup>; Cells (1 mio).

**Minimal Volume:** Plasma / cell culture medium (25 μL); Tissue (10 mg)<sup>1</sup>; Cells (0.5 mio).

**Sample Collection:** Please see our detailed sample collection protocols.

**Quantification:** Absolute, using external calibration.

Please note: For human material, note any known presence of infectious agents

## List of reported compounds

Compound name	Identifier	Formula	Monoisotopic mass
Cysteine	HMDB0000574	C₃H <sub>7</sub> NO₂S	121.020
Glutathione (total)	HMDB0000517	C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>6</sub> S	307.084
γ-Glutamylcysteine	HMDB0001049	C <sub>8</sub> H <sub>14</sub> N <sub>2</sub> O <sub>5</sub> S	250.062
Cysteinylglycine	HMDB0000078	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub> S	178.041

## **LC** conditions

Column	Waters BEH Shield RP 18 50 x 2.1mm		
Temperature	42° C		
Mobile phase A	100 mM K-Acetate, pH 5.3		
Mobile phase B	ACN		
Flow	0.85 ml/min		

<sup>&</sup>lt;sup>1</sup> Pulverized/crushed (deep-frozen) and exact weight noted



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## **Notes**

Samples need to be snap-frozen and stored at -80°C.

Variations in sampling procedures will affect metabolite measurements.

In the majority of sample types analyzed so far the concentrations of  $\gamma$ -Glutamylcysteine and Cysteinylglycine were below limit of detection.

<sup>&</sup>lt;sup>1</sup> Pulverized/crushed (deep-frozen) and exact weight noted