

## ANALYSIS OF AMINO ACIDS

**Description:** UPLC-Fluorescence (FLR) method for the sensitive detection and quantification of amino acids. Detection limits depend on matrix type and input quantity. Samples are extracted using 0.1M HCl, semi-purified, derivatized using AccqTag<sup>®</sup> (Waters) and measured by UPLC-FLR using norleucine as an internal standard for normalization.

Analytes are reported as  $\mu\text{M}$  or pmol/mio cells or pmol/mg tissue.

**Container:** Eppendorf Tube or equivalent

**Optimal Volume:** Plasma / cell culture medium (100  $\mu\text{L}$ ); Tissue (25 mg)<sup>1</sup>; Cells (1 mio).

**Minimal Volume:** Plasma / cell culture medium (25  $\mu\text{L}$ ); Tissue (10 mg)<sup>1</sup>; Cells (0.5 mio).

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

**Quantification:** Absolute, using external calibration and internal std norleucine.

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

### List of reported compounds

(D- and L- enantiomers are not distinguished; for determination of Cys or Trp, see specific methods)

Compound name	Identifier	Formula	Monoisotopic mass
Alanine	<a href="#">HMDB0000161</a>	C <sub>3</sub> H <sub>7</sub> NO <sub>2</sub>	89.048
Arginine	<a href="#">HMDB0000517</a>	C <sub>6</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>	174.112
Asparagine	<a href="#">HMDB0000168</a>	C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub>	132.053
Aspartic Acid	<a href="#">HMDB0000191</a>	C <sub>4</sub> H <sub>7</sub> NO <sub>4</sub>	133.038
Glutamine	<a href="#">HMDB0000641</a>	C <sub>5</sub> H <sub>10</sub> N <sub>2</sub> O <sub>3</sub>	146.069
Glutamic Acid	<a href="#">HMDB0000148</a>	C <sub>5</sub> H <sub>9</sub> NO <sub>4</sub>	147.053
Glycine	<a href="#">HMDB0000123</a>	C <sub>2</sub> H <sub>5</sub> NO <sub>2</sub>	75.032
Histidine	<a href="#">HMDB0000177</a>	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>	155.069
Isoleucine	<a href="#">HMDB0000172</a>	C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>	131.095
Leucine	<a href="#">HMDB0000687</a>	C <sub>6</sub> H <sub>13</sub> NO <sub>2</sub>	131.095
Lysine	<a href="#">HMDB0000182</a>	C <sub>6</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	146.106

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

<b>Methionine</b>	<a href="#">HMDB0000696</a>	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub> S	149.051
<b>Phenylalanine</b>	<a href="#">HMDB0000159</a>	C <sub>9</sub> H <sub>11</sub> NO <sub>2</sub>	165.079
<b>Proline</b>	<a href="#">HMDB0000162</a>	C <sub>5</sub> H <sub>9</sub> NO <sub>2</sub>	115.063
<b>Serine</b>	<a href="#">HMDB0000187</a>	C <sub>3</sub> H <sub>7</sub> NO <sub>3</sub>	105.043
<b>Threonine</b>	<a href="#">HMDB0000167</a>	C <sub>4</sub> H <sub>9</sub> NO <sub>3</sub>	119.058
<b>Tyrosine</b>	<a href="#">HMDB0000158</a>	C <sub>9</sub> H <sub>11</sub> NO <sub>3</sub>	181.0734
<b>Valine</b>	<a href="#">HMDB0000883</a>	C <sub>5</sub> H <sub>11</sub> NO <sub>2</sub>	117.079

#### LC conditions

<b>Column</b>	Waters BEH C18 150 x 2.1mm
<b>Temperature</b>	42° C
<b>Mobile phase A</b>	140 mM Na-Acetate, 7 mM Triethanolamine, pH 6.3
<b>Mobile phase B</b>	ACN
<b>Flow</b>	0.45 ml/min

#### Notes

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

Variations in sampling procedures will affect metabolite measurements.

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