

## ANALYSIS OF TRYPTOPHAN RELATED METABOLITES

**Description:** LC-MS/MS method for the sensitive detection and quantification of tryptophan and its related metabolites. The detection limits depend on matrix type and input quantity. Samples are extracted and measured by a Waters I-Class Plus UPLC System paired with a Sciex QTRAP 6500+.

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

**Container:** Eppendorf Tube or equivalent.

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

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

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

**Quantification:** Absolute, using a >6 point calibration curve and  $r^2 > 98\%$ .

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

### List of analytes reported

(D- and L- enantiomers are not distinguished)

Compound name	Identifier	Formula	Monoisotopic mass
1-methyl-L-Tryptophan	<a href="#">HMDB0243933</a>	C <sub>12</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	218.11
2-Oxo-4-methylthiobutanoic acid	<a href="#">HMDB0001553</a>	C <sub>5</sub> H <sub>8</sub> O <sub>3</sub> S	148.02
3-Hydroxy-Anthranilic acid	<a href="#">HMDB0001476</a>	C <sub>7</sub> H <sub>7</sub> N <sub>3</sub> O <sub>3</sub>	153.04
3-Hydroxy-Kynurenine	<a href="#">HMDB0000732</a>	C <sub>10</sub> H <sub>12</sub> N <sub>2</sub> O <sub>4</sub>	224.08
4-Hydroxyphenylpyruvic acid	<a href="#">HMDB0000707</a>	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	180.04
5-Hydroxyindole-3-acetic acid	<a href="#">HMDB0000763</a>	C <sub>10</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	191.06
5-Methylkynurenine	<a href="#">PubChem10656639</a>	C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>	222.24
Anthranilic acid	<a href="#">HMDB0001123</a>	C <sub>7</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub>	137.05
Dopamine	<a href="#">HMDB0000073</a>	C <sub>8</sub> H <sub>11</sub> N <sub>2</sub> O <sub>2</sub>	153.08
Hydroxyphenyllactic acid	<a href="#">HMDB0000755</a>	C <sub>9</sub> H <sub>10</sub> O <sub>4</sub>	182.06
Indole 3 carboxaldehyde	<a href="#">HMDB0029737</a>	C <sub>9</sub> H <sub>7</sub> NO	145.05
Indole 3 pyruvic acid	<a href="#">HMDB0060484</a>	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	203.06
Indole-3-acetic acid	<a href="#">HMDB0000197</a>	C <sub>10</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub>	175.06
Indole-3-carboxylic acid	<a href="#">HMDB0003320</a>	C <sub>9</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub>	161.05

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

Indole-3-lactic acid	<a href="#">HMDB0000671</a>	C11H11NO3	205.07
Indole-3-propionic acid	<a href="#">HMDB0002302</a>	C11H11NO2	189.08
Kynurenic acid	<a href="#">HMDB0000715</a>	C10H7NO3	189.04
Kynurenine	<a href="#">HMDB0000684</a>	C10H12N2O3	208.08
Melatonin	<a href="#">HMDB0001389</a>	C13H16N2O2	232.12
N-acetyl-5-OH-tryptamine	<a href="#">HMDB0001238</a>	C12H14N2O2	218.11
Neopterin	<a href="#">HMDB0000845</a>	C9H11N5O4	253.08
Nicotinamide	<a href="#">HMDB0001406</a>	C6H6N2O	122.05
Nicotinic acid	<a href="#">HMDB0001488</a>	C6H5NO2	123.03
OXIAA/(2-Oxo-2,3-dihydro-1H-indol-3-yl)acetic acid	<a href="#">PubChem3080590</a>	C10H9NO3	191.18
Phenylpyruvic acid	<a href="#">HMDB0000205</a>	C9H8O3	164.05
Serotonine	<a href="#">HMDB0000259</a>	C10H12N2O	176.09
Tryptamine	<a href="#">HMDB0000303</a>	C10H12N2	160.1
Tryptophan	<a href="#">HMDB0000929</a>	C11H12N2O2	204.09
Xanthurenic acid	<a href="#">HMDB0000881</a>	C10H7NO4	205.04

#### LC conditions

Column	Waters HSS T3 150 x 2.1mm
Temperature	35° C
Mobile phase A	Water + 0.1% formic acid
Mobile phase B	CAN + 0.1% formic acid
Flow	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