Tryptophan and Kynurenine in K\textsubscript{2}-EDTA Human Plasma

(Sensitivity: 40.0 and 10.0 ng/mL)

BMPK has validated a highly sensitive HPLC assay with tandem mass spectrometric detection (LC-MS/MS) for the analysis of tryptophan and kynurenine in K\textsubscript{2}-EDTA human plasma. Tryptophan is an essential amino acid required for protein synthesis and must be obtained through diet. Degradation of tryptophan via the kynurenine pathway ultimately leads to the production of nicotinamide adenine dinucleotide (NAD\textsuperscript{+}). Tryptophan catabolism is also an important factor that has been shown to suppress antitumor immune responses and is found to be up-regulated by some cancers. This assay was used to support a clinical trial at Roswell Park Comprehensive Cancer Center entitled “A Phase I/IIb Study of DEC205mAb-NEY-ESO-1 Fusion Protein (CDX-1401) Given with Adjuvant Poly-ICLC in Combination with INCB024360 for Patients in Remission with Epithelial Ovarian, Fallopian Tube, or Primary Peritoneal Carcinoma Whose Tumors Express NY-ESO-1 or LAGE-1 Antigen A”.

### Specifications and Validation Performance

<table>
<thead>
<tr>
<th>Matrix (Anticoagulant):</th>
<th>Human Plasma (K\textsubscript{2}-EDTA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume:</td>
<td>200 μL</td>
</tr>
<tr>
<td>Preparation Procedure:</td>
<td>Protein Precipitation</td>
</tr>
<tr>
<td>HPLC Column:</td>
<td>C18</td>
</tr>
<tr>
<td>Mobile Phase:</td>
<td>Methanol and Water with Ammonium Formate</td>
</tr>
<tr>
<td>Flow Rate:</td>
<td>500 μL/min</td>
</tr>
<tr>
<td>Detection Type:</td>
<td>Tandem Mass Spectral Analysis (MS/MS)</td>
</tr>
</tbody>
</table>

Calibration Range:
- Tryptophan: 40.0 - 20,000 ng/mL
- Kynurenine: 10.0 - 5,000 ng/mL

Calibrator Accuracy:
- Tryptophan: 99.9% (94.6 - 104%; n=6)
- Kynurenine: 100% (90.5 - 110%; n=6)

Calibrator Precision:
- Tryptophan: 2.05% RSD (0.588 - 3.30%; n=6)
- Kynurenine: 3.67% RSD (1.84 - 6.12%; n=6)

QC Concentrations:
- Tryptophan: 120, 1,400 and 15,000 ng/mL
- Kynurenine: 30.0, 350, and 3,750 ng/mL

QC Accuracy:
- Tryptophan: 101% (97.6 - 103%; n=21)
- Kynurenine: 103% (96.5 - 107%; n=21)

QC Precision:
- Tryptophan: 2.99% RSD (2.08 - 4.44%; n=21)
- Kynurenine: 5.97% RSD (5.12 - 6.91%; n=21)

### Pharmacological Factors of Tryptophan and Kynurenine

#### Biological Function:
Tryptophan is catabolized in tumor tissue by the enzyme indoleamine-2,3-dioxygenase (IDO) to kynurenine and its derivatives via the kynurenine pathway. As these catabolites accumulate they create an immunosuppressive microenvironment providing an opportunity to develop therapies targeting this pathway in an attempt to reverse cancer-induced immunosuppression.

#### Examples of IDO Inhibitors:
- Indoximod, INCB024360, NLG919

---

P. Kalinski (ed.) Tumor Immune Microenvironment in Cancer Progression and Cancer Therapy, Advances in Experimental Medicine and Biology 1036, 2017