

Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

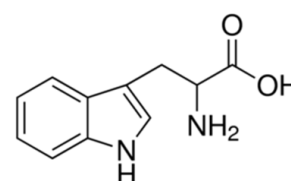
Director: Dr. James Mohler

Tryptophan and Kynurenine in K₂-EDTA Human Plasma (Sensitivity: 40.0 and 10.0 ng/mL)

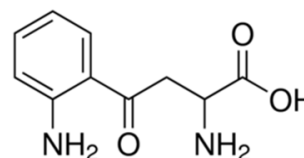
BMPK has validated a highly sensitive HPLC assay with tandem mass spectrometric detection (LC-MS/MS) for the analysis of tryptophan and kynurenine in K₂-EDTA human plasma. Tryptophan is an essential amino acid required for protein synthesis and must be obtained through diet. Degradation of tryptophan via the kynurenine pathway ultimately leads to the production of nicotinamide adenine dinucleotide (NAD⁺). 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-NY-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

Matrix (Anticoagulant):	Human Plasma (K ₂ -EDTA)
Sample Volume:	200 µL
Preparation Procedure:	Protein Precipitation
HPLC Column:	C18
Mobile Phase:	Methanol and Water with Ammonium Formate
Flow Rate:	500 µL/min
Detection Type:	Tandem Mass Spectral Analysis (MS/MS)
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)



Tryptophan
Formula: C₁₁H₁₂N₂O₂
MW: 204.2 g/mol



Kynurenine
Formula: C₁₀H₁₂N₂O₃
MW: 208.2 g/mol

Pharmacological Factors of Tryptophan and Kynurenine¹

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

¹P. Kalinski (ed.) *Tumor Immune Microenvironment in Cancer Progression and Cancer Therapy*, Advances in Experimental Medicine and Biology 1036, 2017

BMPK offers a wide range of bioanalytical and PK/PD modeling services to assist investigators with their basic research, preclinical, and clinical study objectives.

For information on services and pricing, contact John Wilton, Ph.D., Associate Director, at (716) 845-3258 or John.Wilton@RoswellPark.org.

