

# Bioanalytics, Metabolomics and Pharmacokinetics Shared Resource (BMPK)

Director: Dr. James Mohler

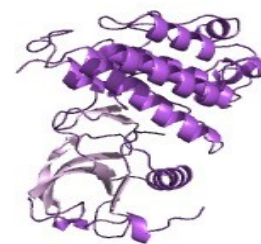
## VEGFR-2 in K<sub>2</sub>EDTA Human Plasma

(Sensitivity: 80.0 pg/mL)

BMPK has recently validated a highly sensitive enzyme-linked immunosorbent assay (ELISA) for the analysis of vascular endothelial growth factor receptor 2 (VEGFR-2) in K<sub>2</sub>EDTA human plasma. The assay was validated using the Quantikine® ELISA Human VEGFR-2/KDR Immunoassay kit system. The preparation procedure is performed using a 96 pin robotic liquid handler to eliminate time dependent variability that may occur with manual pipetting. VEGFR-2, also known as KDR and FLK1 (in mice), is a receptor tyrosine kinase that regulates angiogenesis, vascular development, and embryonic hematopoiesis in response to VEGF isoforms A, C, and D. This assay was used to support clinical trial entitled "Multicenter Phase 1b/2 Study of Tivozanib in Patients with Advanced Inoperable Hepatocellular Carcinoma", which explored the effects of tivozanib, a tyrosine kinase inhibitor, on VEGFR-2 concentrations in human plasma.

### Specifications and Validation Performance

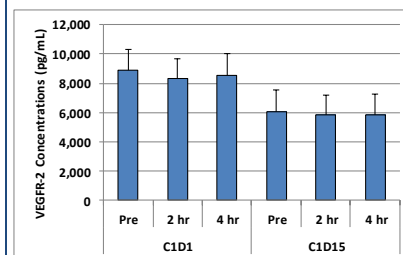
Matrix (Anticoagulant):	Human Plasma (K <sub>2</sub> EDTA)
Required Volume:	50 µL
Preparation Procedure:	ELISA (Solid Phase Sandwich)
Detection Type:	UV (450nm/540nm)
Calibration Ranges:	80.0 - 5,000 pg/mL
Calibrator Accuracy:	98.7% (91.7 - 103%; n=6 runs)
Calibrator Precision:	3.21% CV (0.655 - 8.74%; n=6 runs)
QC Concentrations:	240, 1,000 and 3,750 pg/mL
QC Accuracy:	105% (104 - 105%; n=21 per QC conc)
QC Precision:	7.16% CV (4.88 - 9.25%; n=21 per QC conc)
Assay Stability:	Freeze Thaw Stability: 98.9% mean recovery for 3 different human plasma lots after 3 freeze thaw cycles.  Sample Handling Stability: Stable under yellow lights at room temperature (2.5 hr).  Reanalysis Stability: Sample plate readings are stable for 90 minutes post addition of stop solution.



VEGFR-2

1,356 amino acid residues  
151,527 Daltons

An example of the change in mean VEGFR-2 post drug administration



### Pharmacological Factors of VEGFR-2 <sup>1,2</sup>

Normal Ranges (EDTA Plasma, n=35):

Mean: 9,577 pg/mL (6,400 - 14,500 pg/mL)

Tyrosine Kinase Inhibitors (TKIs):

Tivozanib, sunitinib, sorafenib, and pazopanib are examples of TKIs that bind to various parts of the receptor and prevent interaction with either VEGF or with other downstream signaling molecules.

Biological Function:

VEGFR-2 is a tyrosine kinase receptor responsible for binding with VEGF to initiate signal cascades that stimulate angiogenesis and is a common target for cancer therapy as it is highly up-regulated in many tumor endothelium types.

<sup>1</sup> Quantikine® ELISA Human VEGFR2/KDR Immunoassay Package Insert; <sup>2</sup> Falcon, B.L. et al. Pharmacology & Therapeutics. 164 (2016):204-225.

BMPK offers a wide range of bioanalytical and PK/PD modeling services to assist investigators in 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](mailto:John.Wilton@RoswellPark.org).

