

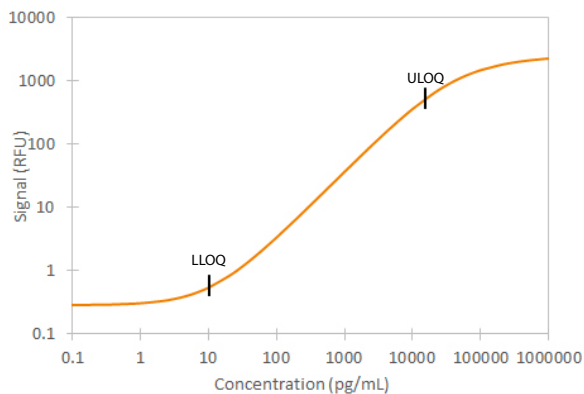
# Human Angiopoietin-2

Simple Plex™ assay for the detection of human Angiopoietin-2 in cell culture supernatant (CCS), serum and plasma (EDTA/Heparin).

## Calibration Curve

The factory generated calibration curve shown below was compiled by averaging replicates of each calibrator from multiple runs. The 4PL curve fit shows calibrator concentration as a function of signal intensity (relative fluorescent units, RFU).

Human serum and plasma assays are quality control tested using a serum/plasma based Bio-Techne Reference Material. Curves may be adjusted to this material for lot-to-lot consistency.



## Limit of Quantitation

Data shown represents typical performance results for Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) of Angiopoietin-2.

	Concentration (pg/mL)
LLOQ	9.91
ULOQ	15,124

## Limit of Detection

The limit of detection (LOD) of human Angiopoietin-2 is 1.3 pg/mL. The LOD was calculated by adding three standard deviations to the mean background signal determined from multiple runs.

## Endogenous Levels

Endogenous levels were calculated from multiple samples. Samples were from apparently healthy volunteers. No medical histories were available for the donors in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	STD DEV (pg/mL)
Serum (n=5)	1419	1096 - 1685	239
EDTA Plasma (n=5)	1114	798 - 1347	199
Heparin Plasma (n=5)	1058	733 - 1345	224

## Precision

**Intra-Assay Precision:** Each control was tested 16 times in one assay.

**Inter-Assay Precision:** Replicates of each control were tested in multiple assays performed by at least three technicians using two lots of reagents.

Parameter	Intra-Assay		Inter-Assay	
	Low	High	Low	High
n	16	16	7	8
Mean (pg/mL)	163	7021	155	7324
Standard Deviation	8.68	454	13.9	298
CV (%)	5.3	6.5	9.0	4.1

## Correlation

This assay has been correlated to the Human Angiopoietin-2 Quantikine™ ELISA Kit (Catalog # DANG20) with an R<sup>2</sup> value greater than 0.9.

## Recovery

Recovery was not necessary as Angiopoietin-2 shows 5 points of natural linearity.

## Linearity

Samples containing and/or spiked with high concentrations of human Angiopoietin-2 were serially diluted with Sample Diluent to produce samples within the dynamic range of the assay.

Dilution	Parameter	CCS (n=6)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Avg % of Expected	91	101	98	94
	Range (%)	84-97	89-106	88-111	87-105
1:4	Avg % of Expected	85	103	105	92
	Range (%)	78-92	98-109	97-114	85-98
1:8	Avg % of Expected	82	104	99	89
	Range (%)	76-92	88-111	79-121	77-104
1:16	Avg % of Expected	83	107	91	89
	Range (%)	72-94	85-124	58-114	74-99

## Specificity

This assay recognizes natural and recombinant human Angiopoietin-2. The factors listed were prepared at 50 ng/mL in Sample Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a rhAngiopoietin-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

- Angiogenin
- Angiopoietin-1
- Angiopoietin-4
- Angiopoietin-like 3
- Angiopoietin-like 4
- Angiopoietin-like 5
- Angiopoietin-like 6
- Angiopoietin-like 7
- Tie-1
- Tie-2
- VEGF-A<sub>111</sub>
- VEGF-A<sub>121</sub>
- VEGF-A<sub>165</sub>
- VEGF-A<sub>145</sub>
- VEGF-A<sub>189</sub>
- VEGF-B<sub>167</sub>
- VEGF-C
- VEGF-D
- VEGF/PlGF
- VEGF R1
- VEGF R2

## Sample Collection and Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Angiopoietin-2 is found in saliva. Take necessary precautions (e.g. mask and gloves) to prevent contamination of the kit reagents while running this assay.**

**Cell Culture Supernatant:** Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** Grossly hemolyzed or icteric samples are not suitable for use with this assay.

## Sample Preparation

Cell culture supernatant samples require a minimum 2-fold dilution with sample diluent. A suggested 2-fold dilution can be achieved by adding 35  $\mu$ L of sample to 35  $\mu$ L of Sample Diluent. Samples above the ULOQ require further dilution.

Serum and plasma samples require a minimum 10-fold dilution with sample diluent. A suggested 10-fold dilution can be achieved by adding 10  $\mu$ L of sample to 90  $\mu$ L of Sample Diluent. Samples above the ULOQ require further dilution.

