

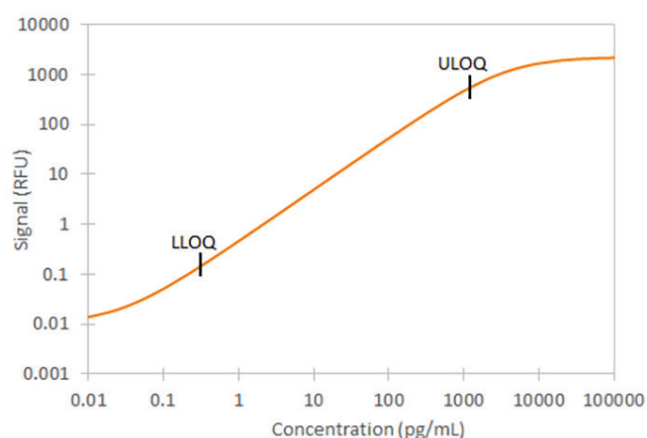
# Non-Human Primate TNF-alpha

Simple Plex™ assay for the detection of non-human primate Tumor Necrosis Factor alpha (TNF-α) 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).

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) for TNF-α.

	Concentration (pg/mL)
LLOQ	0.30
ULOQ	1160

## Limit of Detection

The limit of detection (LOD) of non-human primate TNF-α is 0.03 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.

Sample Type	Mean (pg/mL)	Range (pg/mL)	STD DEV (pg/mL)
Serum (n=10)	5.11	3.92 - 7.16	0.95
EDTA Plasma (n=10)	8.63	2.08 - 46.6	13.5
Heparin plasma (n=10)	10.5	3.30 - 53.4	15.3

## 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
Sample				
n	16	16	36	35
Mean (pg/mL)	6.99	383	7.81	408
Standard Deviation	0.345	20.6	0.662	36.9
CV (%)	4.9	5.4	8.5	9.0

## Recovery

Recovery at three different spiked concentrations within the range of the assay was evaluated.

Sample Type	Average%	Range%
Serum (n=5)	88	78-92
EDTA Plasma (n=5)	86	81-91
Heparin Plasma (n=4)	90	82-97

## Linearity

Samples containing and/or spiked with high concentrations of TNF- $\alpha$  were serially diluted with Sample Diluent to produce samples within the dynamic range of the assay.

Dilution	Parameter	CCS (n=4)	Serum (n=5)	EDTA Plasma (n=5)	Heparin Plasma (n=4)
1:2	Avg % of Expected	94	104	107	104
	Range (%)	90-101	96-108	103-113	101-108
1:4	Avg % of Expected	93	110	111	104
	Range (%)	87-99	101-119	102-116	102-106
1:8	Avg % of Expected	94	110	103	108
	Range (%)	87-102	104-120	100-107	100-117
1:16	Avg % of Expected	95	122	112	110
	Range (%)	87-102	113-137	105-118	104-115

## Specificity

This assay recognizes natural and recombinant TNF- $\alpha$ . The factors listed were prepared at 29 ng/mL in Sample Diluent and assayed for cross-reactivity. Preparations of the following factors at 29 ng/mL in a TNF- $\alpha$  control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

- CD40
- CD40 Ligand
- Fas Ligand
- LIGHT
- TL1A
- TNF- $\beta$
- TNF RI
- TNF RII
- TRANCE
- TRAIL
- TNF- $\alpha$  (pro)

Recombinant human TNF- $\alpha$  cross-reacts approximately 100% in this assay.

Recombinant human pro-TNF- $\alpha$  does not interfere but does cross-react approximately 10% in this assay.

## Sample Collection and Storage

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

**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:** Allow blood samples to clot for 30 minutes at room temperature before centrifuging 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 EDTA or heparin 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

CCS samples require a minimum 5-fold dilution with Sample Diluent. A suggested 5-fold dilution can be achieved by adding 20  $\mu$ L of sample to 80  $\mu$ L of Sample Diluent. Samples above the ULOQ require further dilution.

Serum and plasma 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.

