bio-techne®
R&D SYSTEMS

Specification Sheet

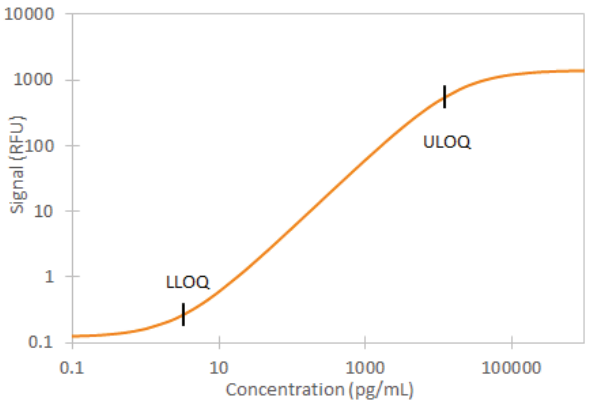
Non-Human Primate Perforin

Simple Plex™ assay for the detection of non-human primate Perforin 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 Perforin.

	Concentration (pg/mL)
LLOQ	3.14
ULOQ	12,000

Limit of Detection

The limit of detection (LOD) of non-human primate Perforin is 0.75 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=8)	3500	2010 - 4900	827
EDTA Plasma (n=8)	2030	1420 - 2740	520
Heparin plasma (n=8)	2750	1640 - 4340	870

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	
Sample	**Low**	**High**	**Low**	**High**
n	16	16	23	25
Mean (pg/mL)	92.9	3901	90.7	4145
Standard Deviation	2.07	74.9	7.41	371
CV (%)	2.2	1.9	8.2	8.9

Recovery

Recovery was not necessary as Perforin shows 5 points of natural linearity.

Linearity

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

Dilution	Parameter	CCS (n=2)	Serum (n=8)	EDTA Plasma (n=8)	Heparin Plasma (n=8)
1:2	Avg % of Expected	96	103	105	106
	Range (%)	94-99	91-114	99-111	97-115
1:4	Avg % of Expected	99	106	110	112
	Range (%)	99-100	93-121	100-124	102-117
1:8	Avg % of Expected	95	112	111	118
	Range (%)	95-96	98-120	94-132	108-153
1:16	Avg % of Expected	96	112	115	116
	Range (%)	96-97	98-134	100-128	109-125

Specificity

This assay recognizes natural and recombinant Perforin. The factors listed were prepared at 330 ng/mL in Sample Diluent and assayed for cross-reactivity. Preparations of the following factors at 330 ng/mL in a Perforin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

- Calreticulin-2
- Granzyme A
- Granzyme B
- Hemopexin

Recombinant human Perforin cross-reacts approximately 100% 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 ≤ -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 ≤ -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 ≤ -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 µL of sample to 80 µL 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 µL of sample to 90 µL of Sample Diluent. Samples above the ULOQ require further dilution.

D10-1329-001 / Rev A