# Human B7 Homolog 1

Simple Plex assay for the detection of human B7-H1/PD-L1 in serum and plasma (EDTA/Heparin)

For research use only. Not for use in diagnostic procedures.

Calibration Curve

The factory generated calibration curve shown below was compiled by averaging 5 replicates of each calibrator from multiple runs. The 5PL curve fit shows calibrator concentration as a function of signal intensity (relative fluorescent units, RFU).
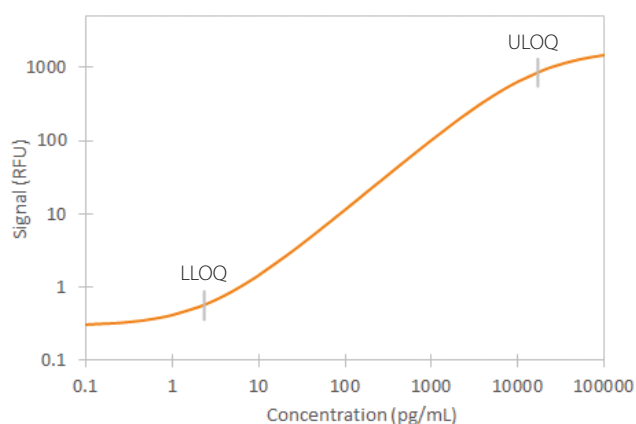
Limit of Quantitation

Data shown represents typical performance results for Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) of B7-H1.

	CONC. (pg/mL)
LLOQ	2.35
ULOQ	17,000

Limit of Detection

The limit of detection (LOD) of human B7-H1 is 0.741 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=10)	198	64.6-790	232
EDTA Plasma (n=10)	188	53.1-752	224
Heparin plasma (n=10)	188	57.2-734	214

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	LOW QC	HIGH QC
Intra-Mean (pg/mL)	86.6	4857
Intra-assay SD	4.71	368
Intra-assay CV (%)	5.4	7.6
Inter-Mean (pg/mL)	88.9	4517
Inter-assay SD	8.69	522
Inter-assay CV (%)	9.8	11.6

Correlation

This assay has been correlated to the Quantikine® ELISA Kit with a slope of 0.9-1.1 and an R^2 value greater than 0.9.

Recovery

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

SAMPLE TYPE	AVG %	RANGE %
Serum (n=4)	111	108-115
EDTA Plasma (n=4)	102	95-108
Heparin Plasma (n=4)	102	94-112

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Linearity

Samples containing and/or spiked with high concentrations of human B7-H1 were serially diluted with Sample Diluent to produce samples within the dynamic range of the assay.

DILUTION	PARAMETER	SERUM (n=4)	EDTA PLASMA (n=4)	HEPARIN PLASMA (n=4)
1:2	Avg % of Expected	94	101	99
	Range (%)	89-100	97-112	89-115
1:4	Avg % of Expected	94	97	94
	Range (%)	88-101	86-110	78-109
1:8	Avg % of Expected	92	97	91
	Range (%)	81-100	85-109	76-111
1:16	Avg % of Expected	91	95	88
	Range (%)	84-97	80-107	70-109

Specificity

This assay recognizes natural and recombinant human B7-H1. 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 rhB7-H1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
- B7-1
- B7-2
- B7-H2
- B7-H3
- B7-H4
- B7-H6
- B7-H7
- PD-1
- PD-L2

Sample Collection and Storage

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

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 ≤ -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 ≤ -20° C. Avoid repeated freeze-thaw cycles.

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

Sample Preparation

Serum and plasma samples require a minimum 2-fold dilution with sample diluent. Samples above the ULOQ require further dilution.

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