## Description

Interferon-alpha (IFN-α) is a group of proteins belonging to the type I interferon family, playing a critical role in the immune response against viral infections. There are multiple subtypes of IFN-α, each encoded by a distinct gene. In humans, the IFN-α family consists of at least 13 subtypes, including IFN-α1, IFN-α2, IFN-α4, IFN-α5, IFN-α6, IFN-α7, IFN-α8, IFN-α10, IFN-α14, IFN-α16, IFN-α17, and IFN-α21 (more variants have been reported).

Molecularly, IFN-α proteins are relatively small, with molecular weights ranging approximately from 19 to 26 kDa. They are produced by leukocytes, primarily in response to viral infections, pathogens and tumor cells. Once secreted, IFN-α binds to the IFN-α/β receptor, triggering a signaling cascade that leads to the expression of interferon-stimulated genes (ISGs). These ISGs encode proteins that inhibit viral replication, enhance the immune system's ability to detect infected cells, and activate immune cells like natural killer cells and macrophages. Physiologically, IFN-α is crucial in the innate immune response, providing an early defense mechanism against viral infections. Due to its immunomodulatory effects, IFN-α has been used in treating Hepatitis C, melanoma and leukemia.

However, the role of IFN-α in disease is complex. While it helps control viral infections and has antitumor properties, its overactivation can contribute to the pathogenesis of autoimmune diseases (SLE, Type1 Diabetes). IFN-α and its subtypes can serve as a biomarker for disease activity, especially in viral infections and certain autoimmune disorders. Monitoring its levels can help assess the severity of the disease and the effectiveness of the treatment. IFN-α MS Advantage PLUS is a digital immunoassay for the quantitative determination of IFN-α in human EDTA plasma and serum.

Calibration Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted. For IFN-α MS Advantage PLUS, the reconstitution volume for calibrator concentrates may vary between kit lots, while keeping the target calibrator concentrations at each level as consistent as possible: The minimum allowable concentration for Cal H is 12 pg/mL and the maximum allowable concentration for Cal B is 0.009 pg/mL.
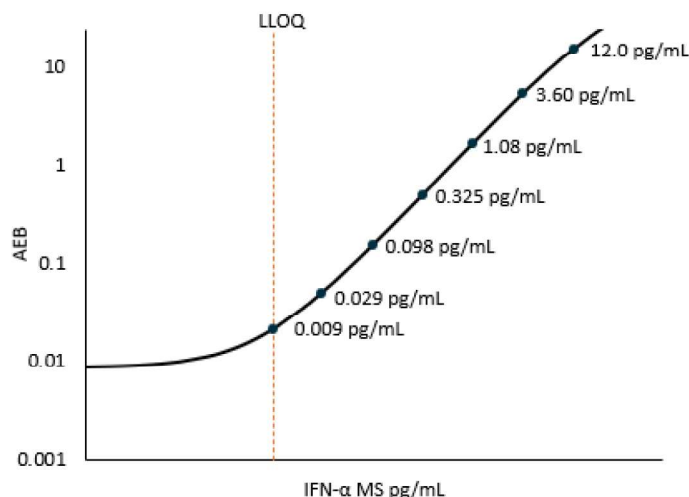
MRD (Minimum Required Dilution)	
Diluted Sample Volume	100 µL per measurement
Human Serum and EDTA Plasma Dilution	1:2
Tests per kit	96

**See Kit Instruction for details.**

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 12 runs each for 2 reagent lots across 2 instruments (3 runs per lot, per instrument). The analytical LLOQ was set at the lowest concentration that read back within 80 − 120% of the expected value with a CV < 20%. The functional LLOQ (fLLOQ) values for each matrix represent the analytical LLOQ multiplied by the dilution factor used for the respective matrix (Table).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean background signal read back on each calibration curve over 12 runs for 2 reagent lots across 2 instruments (3 runs per lot, per instrument).

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The representative ranges below are for serum and EDTA plasma.

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Analytical LLOQ	**0.009 pg/mL** pooled CV 11% mean recovery 99%
Functional LLOQ **(serum and plasma)**	**0.018 pg/mL**
LOD	**0.002 pg/mL** range 0.001–0.003 pg/mL
Dynamic Range **(serum and EDTA plasma)**	**0 – 24 pg/mL**

Endogenous Sample Reading: Concentrations (pg/mL) were determined for matched serum (n=20) and EDTA plasma (n=20) from normal human donors using the IFN-α MS Advantage PLUS kit on HD-X. Bars depict mean with SEM range. The orange line represents functional LLOQ.
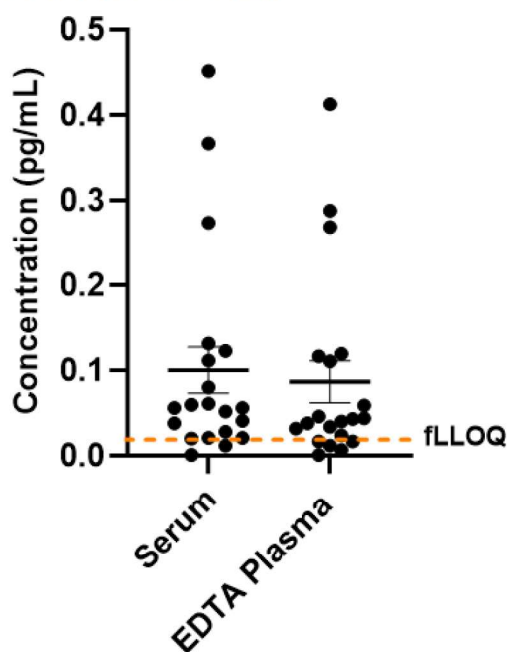
Sample Type	Mean IFN-α MS pg/mL	Median IFN-α MS pg/mL	% Above LOD	% Above LLOQ
Serum	0.111	0.058	95%	90%
EDTA Plasma	0.112	0.046	95%	75%

Precision: Measurements of 2 human serum-based panels, 2 human EDTA plasma-based panels, and 2 calibrator-based controls were measured for precision. Triplicate measurements were made for 6 runs each for 2 reagent lots across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV	Between Lot CV
Control 1	0.193	4%	9%	6%	3%
Control 2	3.80	3%	6%	2%	2%
Panel 1	0.056	4%	11%	3%	5%
Panel 2	0.050	7%	10%	1%	7%
Panel 3	1.50	3%	7%	2%	3%
Panel 4	3.27	2%	7%	1%	3%

Spike and Recovery: Two normal human samples for each matrix (EDTA plasma and serum) were spiked at high (6 pg/mL) and low (1.2 pg/mL) concentrations within the range of the assay and analyzed on HD-X. Percent recovery is defined as the difference between the measured concentration of IFN-α in the spiked sample and the measured concentration in unspiked sample relative to the concentration of IFN-α in spiked calibrator diluent.

Admixture Linearity: Two admixture series for each matrix (serum and EDTA plasma) were prepared by mixing samples with high endogenous IFN-α (high pool) in various ratios with samples of the same matrix with low endogenous IFN-α (low pool). The two admixture series were tested with two reagent lots. The average linearity across the admixture series for the two samples tested with two lots is displayed for each matrix.

Spike and Recovery **(Human Serum)**	**Mean 84%** Range 79–89%
Spike and Recovery **(Human EDTA Plasma)**	**Mean 77%** Range 72–81%
Admixture Linearity **(Human Serum)**	**Mean 111%** Range 96–130%
Admixture Linearity **(Human EDTA Plasma)**	**Mean 102%** Range 83–116%