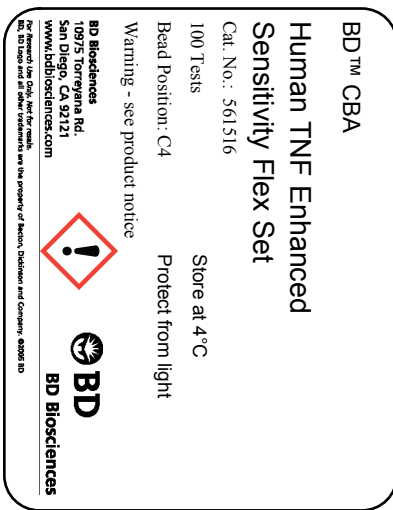


## Technical Data Sheet

## Human TNF Enhanced Sensitivity Flex Set



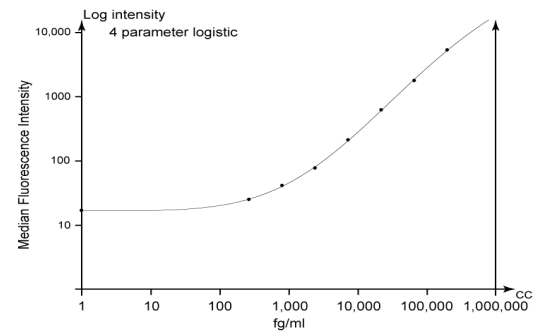
## Product Information

Material Number:	561516
Size:	100 Tests
Bead Position:	C4
Assay Range:	274-200,000 fg/mL
Reactivity:	QC Testing: Human

Component Description: Human TNF Standard  
 Component Mat. No: 51-9003509  
 Component Storage Buffer: Lyophilized in an aqueous buffered solution containing BSA and ProClin™ 150.

Component Description: Human TNF Enhanced Sensitivity Capture Bead C4  
 Component Mat. No: 51-9007209  
 Component Storage Buffer: Aqueous buffered solution containing fetal bovine serum and ≤0.09% sodium azide.

Component Description: Human TNF Detection Reagent (Part A)  
 Component Mat. No: 51-9007210  
 Component Storage Buffer: Aqueous buffered solution containing BSA and ProClin™ 150 as preservative.



**Figure 1. Example BD CBA Human TNF Enhanced Sensitivity Flex Set standard curve.** Data acquired on a BD FACSAria bioanalyzer and analyzed using FCAP Array Software.

## Description

The BD™ CBA Human TNF Enhanced Sensitivity Flex Set is a bead-based immunoassay capable of measuring human Tumor Necrosis Factor (TNF) in serum, plasma, and cell culture supernatant samples. The BD CBA Enhanced Sensitivity Flex Sets are capable of measuring soluble analytes present in very low concentrations. For more information on bead-based immunoassays, refer to the product insert for the BD CBA Human Enhanced Sensitivity Master Buffer Kit (Cat. No. 561521 or 561523).

This BD CBA Enhanced Sensitivity Flex Set contains one vial each of Enhanced Sensitivity Capture Bead and Detection Reagent (Part A) and two vials of Standard. The Enhanced Sensitivity Detection Reagent (Part B) is provided in the BD CBA Human Enhanced Sensitivity Master Buffer Kit. The Enhanced Sensitivity Capture Bead and Detection Reagent (Part A) components of this flex set have been formulated to a 20x concentration to ensure product performance when multiplexed. The Standard component is lyophilized and when reconstituted according to the instructions in the BD CBA Human Enhanced Sensitivity Master Buffer kit, the top standard point for this assay is 200,000 fg/mL. Discard unused reconstituted standard, do not store or reuse. Store lyophilized standard and other components at 4°C. Protect Capture Beads and the Detection Reagent from prolonged exposure to light.

## Application Notes

**Recommended Assay Procedure:** The BD CBA Human TNF Enhanced Sensitivity Flex Set must be used in conjunction with a BD CBA Human Enhanced Sensitivity Master Buffer Kit (Cat. No. 561521, 100 tests, or 561523, 500 tests), a flow cytometer, and FCAP Array™ Software. Detailed instructions on the use of this product can be found in the manual for the BD CBA Human Enhanced Sensitivity Master Buffer Kit. When following the directions in the Master Buffer Kit, the standard range for the BD CBA Human TNF Enhanced Sensitivity Flex Set will be 274 to 200,000 fg/mL. An example standard curve is shown in Figure 1.

The BD CBA Human TNF Enhanced Sensitivity Flex Set should only be used in the same assay well with other BD CBA Human Enhanced

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Sensitivity Flex Set reagents. We do not recommend plating Enhanced Sensitivity Flex Set assays along with assays from any other Flex Set system (soluble protein, cell signaling, etc.). For an updated assay compatibility chart for the BD CBA Human Enhanced Sensitivity Flex Sets, please refer to the BD CBA Flex Set System homepage at <http://www.bdbiosciences.com/cbasetup>.

## Performance

**Limit of Detection:** The theoretical limit of detection is 67.3 fg/mL and was determined by evaluating the estimated result of the average MFI of the negative control (0 fg/mL,  $n=30$ ) + 2 standard deviations.

Specificity		Inter-Assay Reproducibility			Intra-Assay Reproducibility		
		Mean (fg/ml)	Standard Deviation	%CV	Mean (fg/ml)	Standard Deviation	%CV
Human TNF-alpha	Sample 1	2,452.5	154.2	6%	2,416.2	143.6	6%
	Sample 2	7,377.5	235.8	3%	7,524.0	335.6	4%
	Sample 3	21,863.7	943.8	4%	22,641.0	1,067.5	5%

**Reproducibility:** The intra-assay and inter-assay reproducibility were determined for the BD CBA Human TNF Enhanced Sensitivity Flex Set by evaluating ten replicates of three different sample levels (intra-assay) and three replicates of three different sample levels from four separate experiments (inter-assay) respectively.

Specificity	Cell Culture Supernatant		Serum		Plasma	
	Average % Recovery	Range	Average % Recovery	Range	Average % Recovery	Range
Human TNF-alpha	82%	79 - 84%	77%	75 - 79%	80%	77 - 84%

**Recovery:** Cell culture supernatant, serum, or EDTA-treated plasma were spiked with three different levels of protein. The spiked samples were assayed and the results were compared with expected values. Serum and plasma samples were diluted 1:3 before the protein was spiked into each. Serum is a pool of 800 - 1000 donors and the plasma was pooled from at least 20 donors.

Sample Dilution	Cell Culture Supernatant		Serum		Plasma	
	Detected (fg/ml)	% of Expected	Detected (fg/ml)	% of Expected	Detected (fg/ml)	% of Expected
Spiked sample	18,629.2	100%	16,335.8	100%	16,742.3	100%
1 : 3	6,589.3	106%	6,548.0	120%	6,455.0	116%
1 : 9	2,217.0	107%	2,352.3	130%	2,365.3	127%

**Linearity:** Cell culture supernatant, 1:3 diluted serum, or 1:3 diluted EDTA-treated plasma were spiked with protein and serially diluted. The diluted samples were assayed and the results were compared with the original spiked sample.

## Product Notices

- ProClin is a trademark of Rohm and Haas Company.
- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- Warning: CBA lyophilized standard contains 0.02% (w/w) and Detection Reagent (Part A) contains 0.002% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).  
Hazard statement: May cause an allergic skin reaction.  
Precautionary statements: Wear protective gloves/eye protection. Wear protective clothing. Avoid breathing mist/vapours/spray. If skin irritation or rash occurs: Get medical advice/attention. IF ON SKIN: Wash with plenty of water. Dispose of contents/container in accordance with local/regional/national/international regulations.

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