

## Technical Data Sheet Human LT- $\alpha$ Flex Set

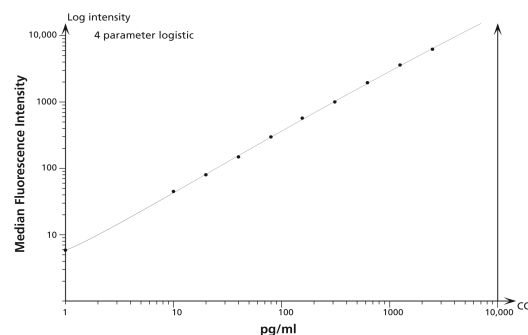
### Product Information

<b>Material Number:</b>	<b>560083</b>
<b>Size:</b>	100 Tests
<b>Bead Position:</b>	D5
<b>Assay Range:</b>	10-2,500 pg/mL
<b>Reactivity:</b>	QC Testing: Human

Component Description: Human LT- $\alpha$  Standard  
 Component Mat. No: 51-9003981  
 Component Storage Buffer: Lyophilized in an aqueous buffered solution containing BSA and ProClin™ 150.

Component Description: Human LT- $\alpha$  PE Detection Reagent  
 Component Mat. No: 51-9004066  
 Component Storage Buffer: Aqueous buffered solution containing BSA and  $\leq 0.09\%$  sodium azide.

Component Description: Human LT- $\alpha$  Capture Bead D5  
 Component Mat. No: 51-9005430  
 Component Storage Buffer: Aqueous buffered solution containing fetal bovine serum and  $\leq 0.09\%$  sodium azide.



**Figure 1. Example BD CBA Human LT- $\alpha$  Flex Set standard curve.** Data acquired on a BD FACSAry bioanalyzer and analyzed using the FCAP Array Software (Cat. No. 641488).

### Description

The BD™ CBA Human LT- $\alpha$  Flex Set is a bead-based immunoassay capable of measuring human lymphotoxin- $\alpha$  (LT- $\alpha$ ), formerly known as tumor necrosis factor- $\beta$ , in serum, plasma, and cell culture supernatant samples. Human reactivity was determined by testing samples with the BD CBA Human LT- $\alpha$  Flex Set. The biology and function of LT- $\alpha$  has been extensively reviewed in the literature. For more information on bead-based immunoassays, refer to the product insert for the BD CBA Human Soluble Protein Master Buffer Kit (Cat. No. 558264 or 558265).

This BD™ CBA Flex Set contains one vial each of Capture Bead and PE Detection Reagent and two vials of Standard. The Capture Bead and PE Detection Reagent components of this flex set have been formulated to a 50x concentration to ensure product performance when multiplexed. The Standard component is lyophilized and should be transferred to a 15 ml polypropylene tube for reconstitution. When reconstituted in 4.0 ml Assay Diluent, the standard has a protein concentration of 2,500 pg/ml. Discard unused reconstituted standard, do not store or reuse. Store lyophilized standard and other components at 4°C. Protect PE Detection Reagent from prolonged exposure to light.

### Application Notes

**Recommended Assay Procedure:** The BD CBA Human LT- $\alpha$  Flex Set must be used in conjunction with a BD CBA Human Soluble Protein Master Buffer Kit (Cat. No. 558264, 100 tests, or 558265, 500 tests), a flow cytometer, and the FCAP Array™ Software (Cat. No. 641488). Detailed instructions on the use of this product can be found in the manual for the BD CBA Human Soluble Protein Master Buffer Kit. When following the directions in the Master Buffer Kit, the top standard point for the BD CBA Human LT- $\alpha$  Flex Set will be 2,500 pg/ml. An example standard curve is shown in Figure 1.

When multiplexing the BD CBA Human LT- $\alpha$  Flex Set assay with the BD CBA Human IL-6 Flex Set assay (Cat. No. 558276) or the BD CBA Human Fas Ligand Flex Set assay (Cat. No. 558330), significantly higher background will be seen in the human IL-6 or Fas Ligand assays. This increased background, while reducing the sensitivity of the human IL-6 or Fas Ligand assay, will not effect the quantitation of human LT- $\alpha$ , Fas

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Ligand or IL-6 in any other way.

The BD CBA Human LT- $\alpha$  Flex Set cannot be used in the same assay well with the following BD CBA Human Soluble Protein Flex Set reagents:

Flex Set	Bead Position	Catalog Number
BD CBA Human IL-3 Flex Set	D5	558335
BD CBA Human VEGF Flex Set	B8	558336
BD CBA Human OSM Flex Set	D5	560084

The BD CBA Human LT- $\alpha$  Flex Set should not be used in the same assay well with any non-BD CBA Human Soluble Protein Flex Set reagents (such as BD CBA Mouse Soluble Protein or Cell Signaling Flex Sets). For an updated assay compatibility chart for the BD CBA Human Soluble Protein Flex Sets, please refer to the BD CBA Flex Set System homepage at <http://www.bdbiosciences.com/flexset>.

### Performance

**Limit of Detection:** The theoretical limit of detection is 0.2 pg/ml and was determined by evaluating the estimated result of the average MFI of the negative control (0 pg/ml,  $n=30$ ) + 2 standard deviations.

Specificity		Inter-Assay Reproducibility			Intra-Assay Reproducibility		
		Mean (pg/ml)	Standard Deviation	%CV	Mean (pg/ml)	Standard Deviation	%CV
Human LT- $\alpha$	Sample 1	40.0	3.4	9%	37.2	2.2	6%
	Sample 2	155.5	6.2	4%	153.2	5.8	4%
	Sample 3	619.8	28.4	5%	595.5	25.1	4%

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

Specificity	Cell Culture Supernatant		Serum		Plasma	
	Average % Recovery	Range	Average % Recovery	Range	Average % Recovery	Range
Human LT- $\alpha$	72%	69 - 73%	74%	71 - 75%	61%	48 - 67%

**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 1:4 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 (pg/ml)	% of Expected	Detected (pg/ml)	% of Expected	Detected (pg/ml)	% of Expected
Spiked sample	521.9	100%	453.0	100%	433.2	100%
1 : 2	272.0	104%	232.2	103%	236.6	109%
1 : 4	139.4	107%	120.7	107%	126.5	117%

**Linearity:** Cell culture supernatant, 1:4 diluted serum, or 1:4 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

1. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
2. ProClin is a trademark of Rohm and Haas Company.
3. 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.

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4. Warning: CBA lyophilized standard contains 0.02% (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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