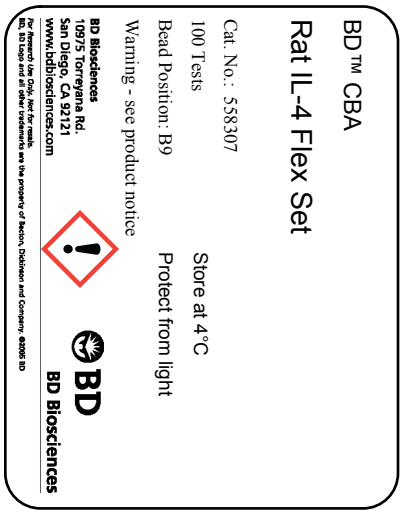

Technical Data Sheet

Rat IL-4 Flex Set

Product Information

Material Number:	**558307**
Size:	100 Tests
Bead Position:	B9
Assay Range:	40-10,000 pg/mL
Reactivity:	QC Testing: Rat

Component Description:	Rat IL-4 Standard
Component Mat. No:	51-9004113
Component Storage Buffer:	Lyophilized in an aqueous buffered solution containing BSA and ProClin™ 150.
Component Description:	Rat IL-4 PE Detection Reagent
Component Mat. No:	51-9004158
Component Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.
Component Description:	Rat IL-4 Capture Bead B9
Component Mat. No:	51-9005246
Component Storage Buffer:	Aqueous buffered solution containing fetal bovine serum and ≤0.09% sodium azide.

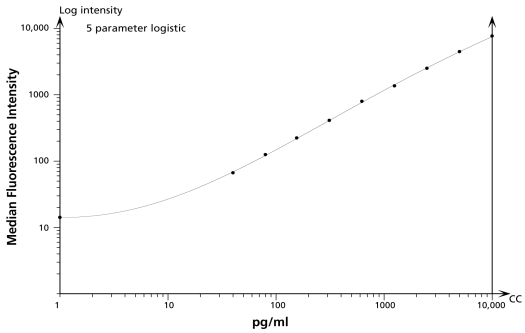

Figure 1. Example BD CBA Rat IL-4 Flex Set standard curve. *Data acquired on a BD FACSArray bioanalyzer and analyzed using FCAP Array Software.*

Description

The BD™ CBA Rat IL-4 Flex Set is a bead-based immunoassay capable of measuring rat interleukin-4 (IL-4) in serum and cell culture supernatant samples. Rat reactivity was determined by testing samples with the BD CBA Rat IL-4 Flex Set. The biology and function of IL-4 has been extensively reviewed in the literature. For more information on bead-based immunoassays, refer to the product insert for the BD CBA Mouse/Rat Soluble Protein Master Buffer Kit (Cat. No. 558266 or 558267).

Preparation and Storage

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 10,000 pg/mL. Discard unused reconstituted standard, do not store or reuse. Store lyophilized standard and other components at 4°C. Protect Capture Beads and the PE Detection Reagent from prolonged exposure to light.

Application Notes

Recommended Assay Procedure: The BD CBA Rat IL-4 Flex Set must be used in conjunction with a BD CBA Mouse/Rat Soluble Protein Master Buffer Kit (Cat. No. 558266, 100 tests, or 558267, 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 Mouse/Rat Soluble Protein Master Buffer Kit. When following the directions in the Master Buffer Kit, the top standard point for the BD CBA Rat IL-4 Flex Set will be 10,000 pg/mL. An example standard curve is shown in figure 1.

When multiplexing the BD CBA Rat IL-4 Flex Set assay with the BD CBA Rat IFN-γ Flex Set assay (Cat. No. 558305), significantly higher

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background will be seen in the rat IL-4 assay. This increased background, while reducing the sensitivity of the rat IL-4 assay, will not effect the quantitation of rat IFN-γ or IL-4 in any other way.

The BD CBA Rat IL-4 Flex Set should not be used in the same assay well with any non-BD CBA Rat Soluble Protein Flex Set reagents (such as BD CBA Human or Mouse Soluble Protein or Cell Signaling Flex Sets). For an updated assay compatibility chart for the BD CBA Rat Soluble Protein 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 3.4 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
Rat IL-4	Sample 1	155.4	9.5	6%		148.2	5.8	4%
	Sample 2	612.6	34.1	6%		606.3	17.0	3%
	Sample 3	2446.0	133.3	5%		2536.1	114.9	5%

Reproducibility: *The inter-assay and intra-assay reproducibility were determined for the BD CBA Rat IL-4 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.*

Sample Dilution	Cell Culture Supernatant		Serum	
	Average % Recovery	Range	Average % Recovery	Range
Rat IL-4	86%	81 - 96%	83%	77 - 92%

Recovery: *Cell culture supernatant and pooled rat serum were spiked with three different levels of protein. The spiked samples were assayed and the results were compared with expected values. Serum samples were diluted 1:4 before the protein was spiked into each. Serum used was commercially available pooled rat serum.*

Sample Dilution	Cell Culture Supernatant		Serum	
	Detected (pg/ml)	% of Expected	Detected (pg/ml)	% of Expected
Spiked sample	2486.5	100%	2282.1	100%
1 : 2	1153.3	93%	1096.6	96%
1 : 4	545.7	88%	539.7	95%

Linearity: *Cell culture supernatant and 1:4 diluted pooled rat serum 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. ProClin is a trademark of Rohm and Haas Company.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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.
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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