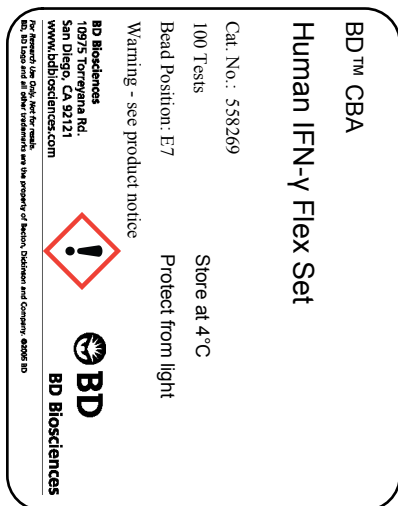


Technical Data Sheet

Human IFN- γ Flex Set

Product Information

| | |
|-------------------------|--|
| Material Number: | 558269 |
| Size: | 100 Tests |
| Bead Position: | E7 |
| Assay Range: | 10-2,500 pg/mL |
| Reactivity: | QC Testing: Human Tested in Development: Rhesus, Cynomolgus, Baboon |



Component Description: Human IFN- γ Standard
 Component Mat. No: 51-9003507
 Component Storage Buffer: Lyophilized in an aqueous buffered solution containing BSA and ProClin™ 150.

Component Description: Human IFN- γ PE Detection Reagent
 Component Mat. No: 51-9004031
 Component Storage Buffer: Aqueous buffered solution containing BSA and $\leq 0.09\%$ sodium azide.

Component Description: Human IFN- γ Capture Bead E7
 Component Mat. No: 51-9005281
 Component Storage Buffer: Aqueous buffered solution containing fetal bovine serum and $\leq 0.09\%$ sodium azide.

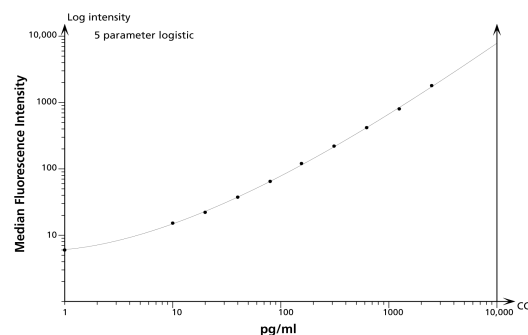


Figure 1. Example BD CBA Human IFN- γ Flex Set standard curve. Data acquired on a BD FACSAry bioanalyzer and analyzed using FCAP Array Software.

Description

The BD™ CBA Human IFN- γ Flex Set is a bead-based immunoassay capable of measuring human interferon- γ (IFN- γ) in serum, plasma, and cell culture supernatant samples. Human and non-human primate reactivity was determined by testing samples with the BD CBA Human IFN- γ Flex Set. The biology and function of IFN- γ 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).

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 2,500 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 Human IFN- γ 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 FCAP Array™ Software. 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 IFN- γ Flex Set will be 2,500 pg/mL. An example standard curve is shown in Figure 1.

The BD CBA Human IFN- γ Flex Set cannot be used in the same assay well with the following BD CBA Human Soluble Protein Flex Set

BD Biosciences

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| United States | Canada | Europe | Japan | Asia Pacific | Latin America/Caribbean |
|---------------|--------------|----------------|--------------|--------------|-------------------------|
| 877.232.8995 | 800.268.5430 | 32.2.400.98.95 | 0120.8555.90 | 65.6861.0633 | 55.11.5185.9995 |

For country contact information, visit bdbiosciences.com/contact

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reagents:

| Flex Set | Bead Position | Catalog Number |
|-------------------------------------|---------------|----------------|
| BD CBA Human IFN- γ Flex Set | B8 | 560111 |

The BD CBA Human IFN- γ 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/cbasetup>.

Performance

Limit of Detection: The theoretical limit of detection is 1.8 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 IFN- γ | Sample 1 | 37.8 | 2.3 | 6% | 34.8 | 1.1 | 3% |
| | Sample 2 | 151.6 | 9.7 | 6% | 138.7 | 3.0 | 2% |
| | Sample 3 | 608.9 | 26.3 | 4% | 576.3 | 12.6 | 2% |

Table 1. Reproducibility: The inter-assay and intra-assay reproducibility were determined for the BD CBA Human IFN- γ 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 IFN- γ | 88% | 81 - 93% | 68% | 55 - 75% | 57% | 45 - 64% |

Table 2. 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 | 538.0 | 100% | 409.9 | 100% | 462.6 | 100% |
| 1 : 2 | 265.8 | 99% | 224.8 | 110% | 249.6 | 108% |
| 1 : 4 | 139.7 | 104% | 117.5 | 115% | 132.1 | 114% |

Table 3. 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. 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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