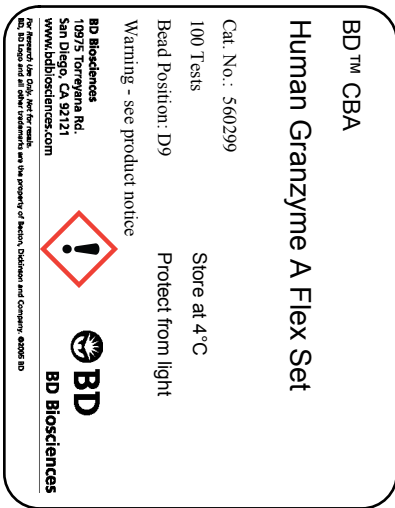


Technical Data Sheet

Human Granzyme A Flex Set



Product Information

Material Number:	560299
Size:	100 Tests
Bead Position:	D9
Assay Range:	40-10,000 pg/mL
Reactivity:	QC Testing: Human

Component Description: Human Granzyme A Standard
 Component Mat. No: 51-9005938
 Component Storage Buffer: Lyophilized in an aqueous buffered solution containing BSA and ProClin™ 150.

Component Description: Human Granzyme A PE Detection Reagent
 Component Mat. No: 51-9005940
 Component Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Component Description: Human Granzyme A Capture Bead D9
 Component Mat. No: 51-9005942
 Component Storage Buffer: Aqueous buffered solution containing fetal bovine serum and ≤0.09% sodium azide.

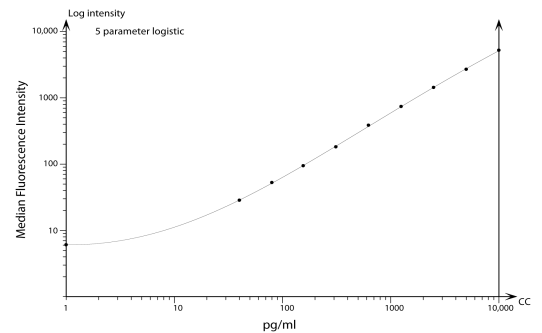


Figure 1. Example BD CBA Human Granzyme A Flex Set standard curve. Data acquired on a BD FACSAry bioanalyzer and analyzed using FCAP Array Software.

Description

The BD™ CBA Human Granzyme A Flex Set is a bead-based immunoassay capable of measuring soluble human Granzyme A in serum, plasma, and cell culture supernatant samples. Human reactivity was determined by testing samples with the BD CBA Human Granzyme A Flex Set. The biology and function of Granzyme A 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 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 Human Granzyme A 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 Granzyme A Flex Set will be 10,000 pg/mL. An example standard curve is shown in Figure 1.

The BD CBA Human Granzyme A Flex Set should not be used in the same assay well with any non-BD CBA Human Soluble Protein Flex Set

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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 3.7 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 Granzyme A	Sample 1	153.3	7.3	5%	142.3	6.2	4%
	Sample 2	569.9	49.7	9%	556.7	27.7	5%
	Sample 3	2525.1	200.6	8%	2605.4	76.9	3%

Reproducibility: The intra-assay and inter-assay reproducibility were determined for the BD CBA Human Granzyme A 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).

Specificity	Cell Culture Supernatant		Serum		Plasma	
	Average % Recovery	Range	Average % Recovery	Range	Average % Recovery	Range
Human Granzyme A	77%	75 - 79%	40%*	39 - 41%*	17%*	15 - 20%*

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.

*The spike recovery for serum and plasma is influenced by the presence of antithrombin III (ATIII) and alpha-2-macroglobulin ($\alpha 2M$), therefore this assay may not be useful for serum and plasma samples. Please see References for more information.

Sample Dilution	Cell Culture Supernatant		Serum		Plasma	
	Detected (pg/mL)	% of Expected	Detected (pg/mL)	% of Expected	Detected (pg/mL)	% of Expected
Spiked sample	1594.4	100%	873.7	100%	436.4	100%
1 : 2	933.4	117%	551.4	126%	283.2	130%
1 : 4	513.4	129%	356.7	163%	157.8	145%

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

- ProClin is a trademark of Rohm and Haas Company.
- Licensed for Research Purposes Only. Commercial use requires license from Boyce Thompson Institute for Plant Research.
- 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) 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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