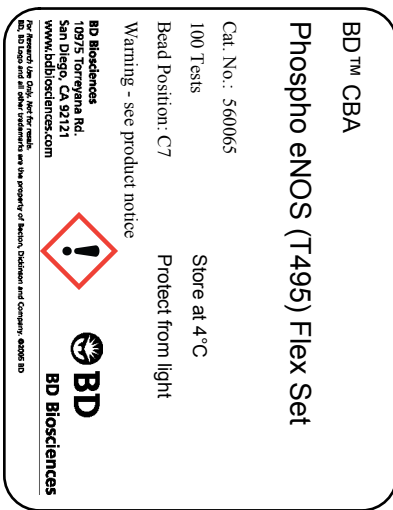


Technical Data Sheet

Phospho eNOS (T495) Flex Set

Product Information

Material Number:	560065
Size:	100 Tests
Bead Position:	C7
Assay Range:	3.9-1,000 Units/mL
Reactivity:	QC Testing: Human



Component Description: Phospho eNOS (T495) Standard
 Component Mat. No: 51-9005394
 Component Storage Buffer: Lyophilized in an aqueous buffered solution containing BSA and ProClin™ 150.

Component Description: Phospho eNOS (T495) PE* Detection Reagent
 Component Mat. No: 51-9005396
 Component Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Component Description: Phospho eNOS (T495) Capture Bead C7
 Component Mat. No: 51-9005397
 Component Storage Buffer: Aqueous buffered solution containing fetal bovine serum and ≤0.09% sodium azide.

Description

The BD™ CBA Phospho eNOS (T495) Flex Set is a bead-based immunoassay capable of measuring human endothelial nitric oxide synthase (eNOS), a cell-type specific enzyme that catalyzes the synthesis of nitric oxide, which has been threonine-phosphorylated at Thr-495 in denatured cell lysate samples. Human reactivity was determined by testing cell lysates with the BD CBA Phospho eNOS (T495) Flex Set. The biology and function of eNOS has been previously reviewed. For more information on bead-based immunoassays, refer to the product insert for the BD CBA Cell Signaling Master Buffer Kit (Cat. No. 560005 or 560006).

Preparation and Storage

This BD™ CBA Flex Set contains one vial of each component listed above. All components of this flex set have been formulated to a 50x concentration to ensure product performance when multiplexed. Store at 4°C. Protect Capture Beads and the PE Detection Reagent from prolonged exposure to light.

The Phospho eNOS (T495) Standard provided in this Flex Set is lyophilized and the standard sphere should be transferred to a 1.5 ml microfuge tube for reconstitution. Reconstitute the standard with 0.1 ml Assay Diluent from the BD CBA Cell Signaling Master Buffer Kit (Cat. No. 560005/560006), warm to 37°C and vortex prior to use. After reconstitution, the standard concentration is 50,000 Units/ml and is stable for 3 months when stored at 4°C. When using reconstituted standard, warm to 37°C and vortex to mix thoroughly before use.

Application Notes

Recommended Assay Procedure: The BD CBA Phospho eNOS (T495) Flex Set must be used in conjunction with a BD CBA Cell Signaling Master Buffer Kit (Cat. No. 560005, 100 tests, or 560006, 500 tests) and a flow cytometer. Detailed instructions on the use of this product can be found in the manual for the BD CBA Cell Signaling Master Buffer Kit. When following the directions in the Master Buffer Kit, the top standard point for the BD CBA Phospho eNOS (T495) Flex Set will be 1,000 Units/ml. An example standard curve is shown in Figure 1.

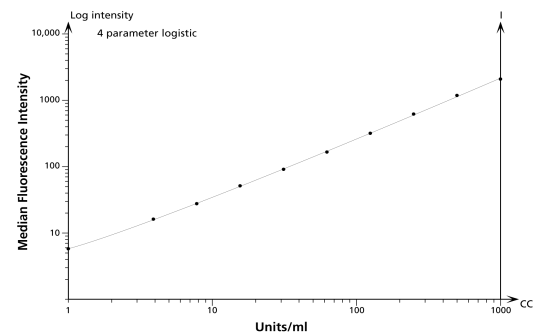


Figure 1. Example BD CBA Phospho eNOS (T495) Flex Set standard curve. Data acquired on a BD FACSAria bioanalyzer and analyzed using the FCAP Array Software (Cat. No. 641488).

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The BD CBA Phospho eNOS (T495) Flex Set cannot be used in the same assay well with the following BD CBA Cell Signaling Flex Set reagents:

Flex Set	Bead Position	Catalog Number
BD CBA Phospho eNOS (S1177) Flex Set	C7	560021
BD CBA Phospho JNK1/2 (T183/Y185) Flex Set	B5	560013

The BD CBA Phospho eNOS (T495) Flex Set should not be used in the same assay well with any non-BD CBA Cell Signaling Flex Set reagents (such as BD CBA Human or Mouse Soluble Protein Flex Sets) **nor with any BD CBA Total Protein Cell Signaling Flex Set reagents**. For an updated assay compatibility chart for the BD CBA Cell Signaling 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 0.9 Units/ml and was determined by evaluating the estimated result of the average MFI of the negative control (0 Units/ml, $n=30$) + 2 standard deviations.

Specificity		Inter-Assay Reproducibility			Intra-Assay Reproducibility		
		Mean (Units/ml)	Standard Deviation	%CV	Mean (Units/ml)	Standard Deviation	%CV
Phospho eNOS (T495)	Sample 1	29.8	1.4	5%	29.9	2.8	9%
	Sample 2	122.9	8.8	7%	116.8	4.7	4%
	Sample 3	490.7	15.0	3%	481.5	15.1	3%

Reproducibility: The inter-assay and intra-assay reproducibility were determined for the BD CBA Phospho eNOS (T495) Flex Set by evaluating ten replicates of three different sample levels (intra-assay) and two replicates of three different sample levels from four separate experiments (inter-assay).

Lysate Dilution	Phospho eNOS (T495)	
	Detected (Units/ml)	% of Expected
Neat	60.0	100%
1 : 2	35.5	118%
1 : 4	19.1	127%
1 : 8	10.0	133%

Linearity: An activated cell lysate was serially diluted to determine the linearity of the assay.

Product Notices

- Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- ProClin is a trademark of Rohm and Haas Company.
- 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 32.07% sodium dodecyl sulfate (w/w) and 0.01% (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 be harmful if swallowed. Causes skin irritation. Causes serious eye irritation. 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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