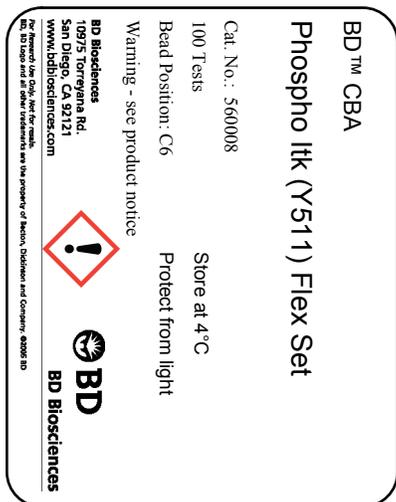


## Technical Data Sheet

### Phospho Itk (Y511) Flex Set

#### Product Information

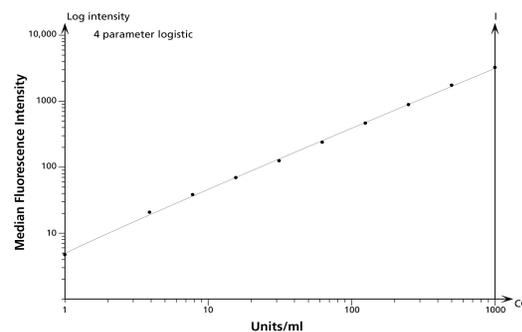
<b>Material Number:</b>	<b>560008</b>
<b>Size:</b>	100 Tests
<b>Bead Position:</b>	C6
<b>Assay Range:</b>	3.9-1,000 Units/mL
<b>Reactivity:</b>	QC Testing: Human Tested in Development: Mouse



Component Description: Phospho Itk (Y511) Standard  
 Component Mat. No: 51-9005175  
 Component Storage Buffer: Lyophilized in an aqueous buffered solution containing BSA and ProClin™ 150.

Component Description: Phospho Itk (Y511) PE\* Detection Reagent  
 Component Mat. No: 51-9005176  
 Component Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

Component Description: Phospho Itk (Y511) Capture Bead C6  
 Component Mat. No: 51-9005178  
 Component Storage Buffer: Aqueous buffered solution containing fetal bovine serum and ≤0.09% sodium azide.



**Figure 1. Example BD CBA Phospho Itk (Y511) Flex Set standard curve.** Data acquired on a BD FACSAry bioanalyzer and analyzed using FCAP Array Software.

#### Description

The BD™ CBA Phospho Itk (Y511) Flex Set is a bead-based immunoassay capable of measuring human or mouse Interleukin-2 inducible tyrosine kinase (Itk), a member of the Tec family of intracellular tyrosine kinases, which has been tyrosine-phosphorylated at Tyr-511 in denatured cell lysate samples. Human reactivity was determined by testing cell lysates with the BD CBA Phospho Itk (Y511) Flex Set. Reactivity with mouse samples was determined by western blot for each of the antibodies used in the BD CBA Phospho Itk (Y511) Flex Set. There are several publications evaluating the biology and function of Itk. 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 Itk (Y511) 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 Itk (Y511) 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 Itk (Y511) Flex Set will be 1,000 Units/mL. An example standard curve is shown in Figure 1.

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The BD CBA Phospho Itk (Y511) 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/cbasetup>.

## Performance

**Limit of Detection:** The theoretical limit of detection is 0.47 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 Itk (Y511)	Sample 1	29.5	1.9	6%	28.3	0.8	3%
	Sample 2	126.6	10.3	8%	114.8	4.0	3%
	Sample 3	509.3	21.1	4%	484.6	12.6	3%

**Reproducibility:** The inter-assay and intra-assay reproducibility were determined for the BD CBA Phospho Itk (Y511) 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 Itk (Y511)	
	Detected (Units/ml)	% of Expected
Neat	65.2	100%
1 : 2	33.2	102%
1 : 4	15.2	93%
1 : 8	7.9	97%

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

## Product Notices

- ProClin is a trademark of Rohm and Haas Company.
- 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 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.

## References

- Siliciano JD, Morrow TA, Desiderio SV. Itk, a T-cell-specific tyrosine kinase gene inducible by interleukin 2. *Proc Natl Acad Sci U S A*. 1992; 89(23):11194-11198. (Biology)
- Wilcox HM, Berg LJ. Itk phosphorylation sites are required for functional activity in primary T cells. *J Biol Chem*. 2003; 278:37112-37121. (Biology)

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