# Technical Data Sheet

# V450 Rat Anti-Mouse IL-2

### **Product Information**

Material Number:	
Size:	
Concentration:	
Clone:	
Immunogen:	
Isotype:	
Reactivity:	
Storage Buffer:	

560546 50 μg 0.2 mg/ml JES6-5H4 Mouse IL-2 Recombinant Protein Rat IgG2b QC Testing: Mouse Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

### Description

The JES6-5H4 monoclonal antibody specifically binds to mouse interleukin-2 (IL-2). IL-2 is a multifunctional cytokine that plays pivotal roles in immunity and tolerance. It is produced by activated T cells. IL-2 effects the activation, growth, proliferation and/or differentiation of various cell types including T and B lymphocytes and their precursors, LAK cells, NK cells, and monocytes/macrophages. IL-2 mediates its biological activities by binding to IL-2 receptor compleses. The intermediate affinity IL-2R is comprised of IL-2R $\beta$  (CD122) and common gamma chain ( $\gamma$ c; CD132) subunits whereas the high-affinity IL-2R is comprised of IL-2R $\beta$  and  $\gamma$ c subunits. The JES6-5H4 monoclonal antibody binds to IL-2 and neutralizes its biological activity.

The antibody is conjugated to BD Horizon<sup>TM</sup> V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the Violet laser Ex max of 406 nm and has an Em Max at **450** nm. Conjugates with BD Horizon<sup>TM</sup> V450 can be used in place of Pacific Blue<sup>TM</sup> conjugates.



Flow cytometric analysis for IL-2 in activated mouse splenocytes. Mouse Intracellular Cytokine-1 positive control cells (MiCK-1) offered by BD Biosciences as MN 554652, are activated mouse splenocytes prepared in the presence of a protein transport inhibitor. Fixed and permeabilized MiCK-1 cells were stained either with a BD Horizon™ V450 Rat IgG2b, κ isotype control (left panel) or with the BD Horizon™ V450 Rat Anti-Mouse IL-2 antibody (right panel). Dot plots were derived from gated events based on light scattering characteristics for lymphocytes. Flow cytometry was performed on a BD LSR™ II flow cytometry system.

## Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon<sup>TM</sup> V450 under optimum conditions, and unreacted BD Horizon<sup>TM</sup> V450 was removed.

### **Application Notes**

Application						
	Intracellular staining (flow cytometry)	Routinely Tested				
R	ecommended Assay Procedure:					

*Flow cytometry:* The JES6-5H4 antibody is useful for immunofluorescent staining and flow cytometric analysis to identify and enumerate IL-2 producing cells within mixed cell populations. A useful control investigators may consider using for demonstrating specificity of staining, is to pre-block with one of the following reagents: (1) recombinant mouse IL-2 (Cat. No. 550069) or with (2) unlabeled JES6-5H4 antibody (Cat. No.

## **BD Biosciences**

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*Cell Preparation:* Investigators not wishing to utilize MiCK-1 cells may alternatively prepare mouse splenocytes (e.g BALB/c) stimulated for 4-6 hours with PMA (5 ng/mL, Sigma-Aldrich Cat. No. P-8139) and ionomycin (500 ng, Sigma-Aldrich Cat. No. I-0634) in the presence of 1 µg/mL Brefeldin A (BD GolgiPlug<sup>TM</sup> MN 555029). Investigators are advised to fix and permeabilize the cells prior to staining.

### Suggested Companion Products

Catalog Number	Name	Size	Clone	
560457	V450 Rat IgG2b, к Isotype Control	0.1 mg	A95-1	
550069	Recombinant Mouse IL-2	20 µg	(none)	
554425	Purified Rat Anti-Mouse IL-2	0.1 mg	JES6-5H4	
554652	MiCK-1 Mouse Cytokine Positive Control Cells	1.0 ml	(none)	
553142	Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block <sup>™</sup> )	0.5 mg	2.4G2	
555028	BD Cytofix/Cytoperm Plus Kit (with BD GolgiPlug)	250 tests	(none)	
554715	BD Cytofix/Cytoperm Plus Kit (with BD GolgiStop)	250 tests	(none)	
555029	Protein Transport Inhibitor (Containing Brefeldin A)	1.0 ml	(none)	

#### **Product Notices**

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2. An isotype control should be used at the same concentration as the antibody of interest.
- BD Horizon<sup>™</sup> V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
- 4. Pacific Blue<sup>TM</sup> is a trademark of Molecular Probes, Inc., Eugene, OR.
- 5. 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.
- 6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
- 7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

#### References

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