





## THE ELISPOT Source Elevating T Cell Multiplexing to New Heights with Four Color FluoroSpot

ptimized ELISPOT/FluoroSpot assays can reliably detect antigen-specific T cells even if they occur in frequencies of 0.0001% within PBMC. That's a single analyte-secreting T cell that can be reliably identified within one million bystander cells. To further increase the use of precious cell material, we offer the most comprehensive portfolio of multicolor ELISPOT kits available on the market, including two-, three-, and four-color T cell kit. Multiplexing analytes not only helps economize cell utilization but it also enables the detection of polyfunctional T cells.



The reagents included in the fluorescent ImmunoSpot<sup>®</sup> Kits have been tailored to shorten and simplify the assay procedure. Kits contain low auto-fluorescent plates, diluents, serum-free media, antibodies, and most importantly, fluorescent conjugates that have separate emission spectrums to easily and accurately detect up to four cytokines. ELISPOT assays have never been faster and easier!

# **IMMUN SPOT** Multiplexing T Cell FluoroSpot and ELISPOT

## The First Step in Multiplexing: Double-Color Kits

CTL is taking multiplexing of T cell assays even further with the only double-color enzymatic ELISPOT Kit on the market that can reliably and accurately count polyfunctional cells. ELISPOT is known to be able to detect a single secreting cell within a million bystander cells. All ImmunoSpot® Kits, human, non-human primate, mouse, and porcine kits, are quality-controlled to meet this exquisite sensitivity threshold.



ImmunoSpot<sup>®</sup> Kits were designed to minimize common assay problems associated with unspecific enhancement or suppression. CTL-Test<sup>™</sup> Medium is a standardized, serumfree alternative to the lot-to-lot variation experienced with serum-containing media, which is known to interfere with the reliability of test results. ImmunoSpot® Kits can be used in conjunction with ePBMC<sup>®</sup> Reference Sample QC Sets<sup>™</sup> to reduce the intra- and inter-laboratory variability.

Double-Color Enzymatic Kits come with two substrates that precisely resolve the quantity of analyte produced by each cell, permitting accurate gating and data analysis. The CTL substrate technology prevents overdevelopment, which allows adjacent spots in the most crowded wells to be resolved. This critical component enables fixed development times, avoiding the subjective judgment calls as to the best time to stop the reaction when using common substrates. Most importantly, Double-Color ImmunoSpot<sup>®</sup> Kits come with two substrates, CTL-TrueBlue<sup>™</sup> and CTL-TrueRed<sup>™</sup>, which are far enough apart on the RGB color spectrum to detect singlecolor spots as well as double-color polyfunctional spots.



**It's <u>THE ONLY</u> Complete Kit** ImmunoSpot® Kits contain everything needed to reliably detect cell secretions. Every Kit contains CTL Serum-free Media, plates, antibodies, and diluents. Enzymatic ImmunoSpot® Kits have enzymatic conjugates with corresponding substrates that have established development times, and double-color kits have two substrates with RGB color values on opposite ends of the spectrum, all in order to allow optimal analysis of double-secreting cells. Fluorescent ImmunoSpot® Kits have fluorescent conjugates to which the fluorochromes have separate emission spectrums. This allows both single analyte-secreting cells and polyfunctional cells to be easily detected.

### SINGLE-COLOR ENZYMATIC KITS & SINGLE-COLOR FLUORESCENT KITS \*pre-coated plates available

HUMAN						
IFN-γ *	IL-2*	IL-4*	IL-5*	IL-6*	IL-10*	IL-12*
IL-13*	IL-17*	IL-21	IL-22	TNF-α*	GM-CSF*	GzB*
Perforin						
NHP						
IFN-γ *	IL-2*	IL-4*	IL-5*	IL-6*	IL-10*	IL-12*
IL-13*	IL-17*	TNF-α*	GM-CSF*	Perforin		
MOUSE						
IFN-γ*	IL-2*	IL-4*	IL-5*	IL-10*	IL-17*	TNF-α*
PIG						
IFN-γ						
RAT						
IFN-γ						

### DOUBLE-COLOR ENZYMATIC KITS & DOUBLE-COLOR FLUORESCENT KITS \*pre-coated plates available for fluorescent only

HUMAN						
IFN-γ / IL-2*	IFN-γ / IL-4*	IFN-γ / IL-5*	IFN-γ / IL-6*	IFN-γ / IL-10*	IFN-γ / IL-12*	IFN-γ / IL-13*
IFN-γ / IL-17*	IFN-γ / IL-21	IFN-γ / IL-22	IFN-γ / GM-CSF*	IFN-γ / GzB*	IFN-γ / Perforin	IFN-γ / TNF-α*
IL-5 / IL-17*	IL-10 / IL-12*	IL-10 / IL-17*	GzB / IL-2*	GzB / Perforin	GzB / TNF-α*	TNF-α / IL-2*
TNF-α / Perforin	IL-4 / IL-5*					
NHP						
IFN-γ / IL-2*	IFN-γ / IL-4*	IFN-γ / IL-5*	IFN-γ / IL-6*	IFN-γ / IL-10*	IFN-γ / IL-12*	IFN-γ / IL-13*
IFN-γ / IL-17*	IFN-γ / GM-CSF*	IFN-γ / Perforin	IFN-γ / TNF-α*	IL-5 / IL-17*	IL-10 / IL-12*	IL-10 / IL-17*
TNF-α / IL-2*	TNF-α / Perforin	IL-4 / IL-5*				
MOUSE						
IFN-γ / IL-2*	IFN-γ / IL-4*	IFN-γ / IL-5*	IFN-γ / IL-10*	IFN-γ / IL-17*	IFN-γ / TNF-α*	IL-2 / TNF-α*
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## THREE-COLOR FLUORESCENT KITS \*pre-coated plates available

HUMAN						
IFN-γ / IL-2 / IL-21	IFN-γ / IL-2 / GzB*	IFN-γ / IL-2 / Perforin	IFN-γ / IL-5 / IL-17*	IFN-γ / IL-10 / IL-4*	IFN-γ / IL-10 / IL-5*	IFN-γ / IL-10 / IL-6*
IFN-γ / IL-10 / IL-12*	IFN-γ / IL-10 / IL-17*	IFN-γ / GzB / IL-5*	IFN-γ / GzB / IL-10	IFN-γ / GzB / IL-17	IFN-γ / GzB / Perforin	IFN- $\gamma$ / TNF- $\alpha$ / IL-2*
IFN-γ / TNF-α / IL-6*	IFN-γ / TNF-α / IL-10*	IFN-γ / TNF-α / IL-17*	IFN-γ / TNF-α / GzB*	IFN- $\gamma$ / TNF- $\alpha$ / Perforin	IL-10 / TNF-α / IL-5	IL-10 / TNF-α / IL-17
IFN / IL-4 / IL-5*						
NHP						
IFN-γ / IL-2 / Perforin	IFN-γ / IL-5 / IL-17*	IFN-γ / IL-10 / IL-4*	IFN-γ / IL-10 / IL-5*	IFN-γ / IL-10 / IL-6*	IFN-γ / IL-10 / IL-12*	IFN-γ / IL-10 / IL-17*
IFN-γ / TNF-α / IL-2*	IFN-γ / TNF-α / IL-6*	IFN-γ / TNF-α / IL-10*	IFN-γ / TNF-α / IL-17*	IFN- $\gamma$ / TNF- $\alpha$ / Perforin	IL-10 / TNF-α / IL-5	IL-10 / TNF-α / IL-17
IFN / IL-4 / IL-5*						
MOUSE						
IFN-γ / IL-4 / IL-17	IFN-γ / TNF-α / IL-2*	IFN-γ / TNF-α / IL-10	IFN-γ / TNF-α / IL-17			

FOUR-COLOR FLUORESCENT KITS \*pre-coated plates available

HUMAN				
IFN-γ / IL-2 / IL-5 / IL-17*	IFN-γ / IL-2 / IL-5 / GzB*	IFN-γ / GzB / IL-2 / Perforin	IFN-γ / TNF-α / IL-2 / GzB*	IFN-γ / TNF-α / IL-2 / Perforin
NHP				
IFN-v / II -2 / II -5 / II -17*	IEN-v / TNF-a / II -2 / Perforin			

CYTOKINE / ANTIBODY FLUORESCENT KITS								
HUMAN								
IFN-γ / IgG / IgA	IFN-γ / IgG / IgM	IL-6 / IgG / IgA	IL-6 / IgG / IgM	IL-10 / IgG / IgA	IL-10 / IgG / IgM	TNF-α / IgG / IgA		
TNF-α / IgG / IgM	GzB / IgG / IgA	GzB / IgG / IgM						
NHP								
IFN-γ / IgG / IgA	IFN-γ / IgG / IgM	IL-6 / IgG / IgA	IL-6 / IgG / IgM	IL-10 / IgG / IgA	IL-10 / IgG / IgM	TNF-α / IgG / IgA		
TNF-α / IgG / IgM								
PORCINE								
IFN-γ / IgA								

# **IMMUNeSPOT** Multiplexing T Cell FluoroSpot and ELISPOT

ImmunoSpot <sup>®</sup> Kits versus the Competition							
Features and Benefits	ImmunoSpot®	COMPETITOR 1	COMPETITOR 2	COMPETITOR 3	COMPETITOR 4	COMPETITOR 5	
96-well plate option	~	~	<b>v</b>	<ul> <li>✓</li> </ul>	~	~	
Strip-plate option	~	<b>v</b>					
384-well plate option	~						
Low auto-fluorescence plate option	~	<ul> <li>✓</li> </ul>	<b>v</b>				
Pre-coated plate option	~	<b>v</b>			<ul> <li></li> </ul>	~	
Serum-free media	~						
Diluents	~		<b>v</b>				
Wash buffer concentrate			<ul> <li>✓</li> </ul>	<ul> <li>✓</li> </ul>			
Coating antibody	~	~	<b>v</b>	<ul> <li>✓</li> </ul>	~	~	
Detection antibody	~	~	<ul> <li>✓</li> </ul>	<ul> <li>✓</li> </ul>	<b>v</b>	~	
Enzymatic or Fluorescent Conjugate	~	~	<ul> <li>✓</li> </ul>	<ul> <li>✓</li> </ul>	~	~	
Single-color substrate	~	<b>v</b>	<ul> <li>✓</li> </ul>	<ul> <li>✓</li> </ul>	<b>v</b>	<b>v</b>	
Optimally calibrated substrates for dou- ble-color enzymatic assays	~						
No blocking step	~						
No artificial fluorescent enhancers	~						
T cell and B cell kit formats	~	<b>v</b>		<b>v</b>	<b>v</b>		
Cytokine Antibody Kits	~						
Single-color enzymatic T cell kits	~	~	<ul> <li>✓</li> </ul>	~	~	~	
Single-color enzymatic B cell kits	~	<b>v</b>		<b>v</b>	<b>v</b>		
Double-color enzymatic T cell kits	~		<ul> <li>✓</li> </ul>		~	~	
Double-color enzymatic B cell kits	~				<b>v</b>		
Single-color fluorescent kits	~						
Cytokine antibody kits	~						
Double-color fluorescent kits (T cell and B cell)	74	28	8	10	8	0	
Triple-color fluorescent kits (T cell and B cell)	63	10	0	0	0	0	
4-color fluorescent kits (T cell and B cell)	9	2	0	0	0	0	
Characterized PBMC for antigen-specific controls	V						
Kit developed by same company manufacturing ELISPOT reader	V						
Scientific and technical support for assay, analyzer, software and kits	V						



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