C.T.L. Serum-free Media

For Low Background and High Signal in T Cell and B Cell Assays

Even carefully selected serum batches are one-of-a-kind reagents. Unique levels of bioactive molecules in different serum batches affect the baseline activation level (background) and the magnitude of antigen-induced T cell and B cell activation (signal). The CTL Serum-free Media portfolio was developed for low background and high signal to outperform the best sera, and are quality-controlled for optimal and consistent performance in T cell and B cell assays.

Primary cells need the multitude of growth and nutrition factors contained within serum to survive and function. T cells and antigen-presenting cells (APC) are particularly vulnerable and dependent on these factors when freshly isolated from blood, tested in vitro, cryopreserved, or thawed. In the past, carefully selected sera were needed to support PBMC during isolation, testing, freezing, and thawing. No longer!

Low Background

Low background, or ideally no background at all, is critical for PBMC-based T cell assays because rare antigen-specific T cells need to be detected amongst all other cells. The frequency of antigen-specific T cells seldom reaches one within a thousand PBMC, and often is as low as one within a hundred thousand, and less. Only in low-background assays can such low-frequency T cells be reliably detected. Each batch of CTL Serum-free Media is quality controlled for low-background activity, typically providing zero to five non-specific IFN-γ, IL-2, IL-4, IL-5 and IL-17 ELISPOTs in PBMC of healthy donors. Serum, in contrast, frequently causes an elevated background *(Figure 1A)*. Even brief exposure of PBMC to serum with mitogenic properties, e.g., during cryopreservation, or when washing the cells, can result in a high background. Entire T cell monitoring trials have been lost because PBMC were either frozen or tested with mitogenic serum!

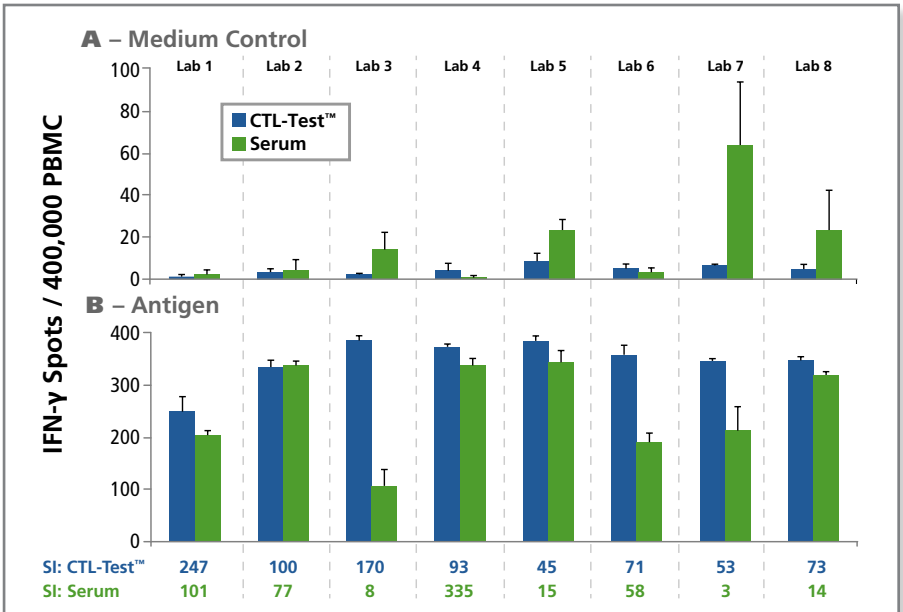

***Figure 1:* Performance of serum-free CTL Test™ Medium compared with eight different qualified sera.** Cryopreserved PBMC of the same batch were thawed and tested in an IFN-γ ELISPOT assay in eight different laboratories using either CTL-Test™ (blue), or, in parallel, in the serum that the respective laboratory has selected for T cell work (green). Spot counts obtained in the medium control are shown in **Panel A.** The antigen- (CEF peptide)- induced spot counts are shown in **Panel B.** The stimulation index (SI: Antigen-induced spots/medium background) defining the strength of signal measured is also shown for each of the laboratories for the results obtained with CTL-Test™ (SI: CTL-Test™), and with the respective laboratory's serum (SI: Serum). In all cases, the maximal spot counts with CTL-Test™ were equal to better than the sera, and four of the eight sera induced an elevated background (Zhang et al., *J. Immunotoxicology,* 2009, 6:227).

High Signal

Serum always contains suppressive factors, such as TGFβ and IL-10. If present in increased concentrations, such molecules inhibit T cell activation, jeopardizing the detection of antigen-specific T cells in functional assays. For detecting antigen-specific CD8 cells, CTL Serum-free Medium matches or outperforms antigen-induced ELISPOT counts elicited with sera that have been qualified for T cell assays *(Figure 1B)*. For CD4 cell detection, frequently

C.T.L. Serum-free Media

remarkable increases in antigen-induced spot counts are seen with CTL Serum-free Media *(Figure 2)*. This surprising finding is consistent with the notion that CD4 cells are more susceptible to suppressive serum cytokine effects than CD8 cells.

Standardization and Quality Control

Using different sera while working with PBMC is likely to produce different results. Each serum is a unique reagent containing unique concentrations of molecules that activate or inhibit T cells and APC. The need to work with serum has been a major obstacle for standardized and reproducible ex vivo experimentation. No longer! CTL Serum-free Media contain highly-defined and constant bioactive components, and each batch is quality controlled for consistant performance. The CTL Serum-free Media platform permits assay standardization within a laboratory, and even across laboratories *(Figure 1)*.

Cost Effective

Consider the expense of working with serum-containing media, which far surpasses the cost of the serum itself. Add to that the work and materials involved in testing different batches to select the best performing one, then ordering in bulk quantities, taking up substantial freezer space to store long-term, and then preparation and sterile filterization of your self-made media and you have a substantial investment of time and money. Ready-to-use CTL Serum-free Media is a cost-effective, and hassle-free alternative.

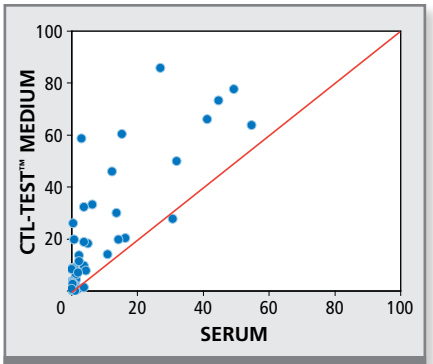

Figure 2. **IFN-γ ELISPOT assay performance with serum-containing complete RPMI medium vs. the serum-free CTL-Test™ Medium.** Freshly isolated PBMC of three healthy donors were tested in parallel under both conditions; the number of spots obtained using CTL-Test™ (Y axis) or in serum (X axis) after stimulation with antigens that stimulate CD4 cells, Mumps, PPD and Candida, is represented by the dots. The red line indicates equal performance. The medium background was zero for all conditions. Note, several recall responses that were not detectable in serum became clearly positive in CTL Test™ Medium.

CTL SERUM-FREE MEDIA PORTFOLIO

CTL has developed Serum-free Media to support all stages of work with PBMC, securing low-background and high-signal detection of T cells and B cells. The members of the serum-free portfolio are:

CTL-Test™: For direct ex vivo testing of PBMC in T cell cytokine assays. (ELISPOT, ELISA, CBA, CPA). (Cat. #CTLT-010, 100ml; #CTLT-005, 500ml)

CTL-Test™ PLUS: Facilitates detection of rare antigen-specific Th1, Th2, and Th17 memory cells. (Cat. #CTLTP-010, 100ml; #CTLTP-005, 500ml)

CTL-Test™ B: Formulated for expansion and ex vivo testing of PBMC in B cell ELISPOT and ELISA assays. (Cat. #CTLTB-010, 100ml; #CTLTB-005, 500ml)

CTL-Wash™ Supplement 10x: Nutrient-rich supplement for PBMC to maintain full viability and functionality (Cat. #CTLW-010, 100ml)

CTL-Cryo™: Serum-free freezing media kit for cryopreservation of freshly-isolated PBMC. (Cat. #CTLC-ABC)

CTL Anti-Aggregate Wash™ 20x: Stabilizes PBMC during thawing; prevents cell loss due to aggregation. (Cat. #CTLAA-005, 5ml, #CTLAA-001, 1ml)

C.T.L.

Cellular Technology Limited

20521 Chagrin Boulevard • Shaker Heights, Cleveland, OH 44122-5350 USA

+1 216-791-5084 • +1 216-751-1373 Fax • +1 888-791-4005 Toll Free US • info@immunospot.com • www.immunospot.com