Bio-Plex® suspension array system

tech note 5927

Multiplexing Across Cytokine Panels: Bio-Plex Pro™ Human and Mouse Group I and Group II

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Introduction

Cytokines, chemokines, and growth factors play an important role in a wide range of physiological processes including immune response, inflammation, and hematopoiesis. They have great utility as biomarkers for a spectrum of disease states. Bio-Rad's Bio-Plex Pro cytokine assays are designed to rapidly quantitate these proteins in diverse matrices such as serum, plasma, and tissue culture supernatant.

The cytokine assay menu includes 48 human and 32 mouse assays that are available in distinct panels: human group I and II and mouse group I and II. These assays are based on magnetic beads, which allow automation of wash steps (using the Bio-Plex Pro wash station), and simplify the assay process by eliminating the need for manual washes on a vacuum manifold. The assays are configured into all-in-one kits that contain the required buffers and diluents. Detailed information about the magnetic bead–based cytokine panels has been described previously (see bulletins 5800 and 5803).

The effectiveness of immunoassays depends on the analytical sensitivity and specificity of the antigen-antibody reaction for the analyte. In this article, the cross-reactivity of antibodies within each species (human or mouse) is described. We show that the majority of human group I and II cytokines may be combined in a 48-plex assay, and all analytes within mouse groups I and II may be combined into a 32-plex assay.

Methods

To evaluate the ability to combine group I and II assays, human group I (27-plex) and group II (21-plex) analytes were tested individually and in combination as a 48-plex. Similarly, mouse group I (23-plex) and group II (9-plex) analytes were tested individually and in combination as a 32-plex.

Evaluation of Assay Specificity

The assay specificity when mixing the analytes from the two assay groups was determined by examining the ratio of background median fluorescence intensity (MFI) of group I and II assays when tested alone and in combination. Background MFI is the signal obtained in the absence of specific binding and is attributable to assay noise such as instrument and reagent noise. It was determined in the presence of multiplexed beads coupled to capture antibodies and detection antibodies in standard diluent lacking the standard proteins. Specificity within each assay group was also established in single detection antibody cross-reactivity experiments for individual analytes.

Evaluation of Assay Sensitivity

The impact on the assay sensitivity upon mixing of analytes from the two assay groups was determined by comparing the differences in the limit of detection (LOD) between individual and combined panels.
LOD is defined as the concentration of analyte (measured in the standard diluent) from the standard curve for which the corresponding MFI is two standard deviations above the background.

Evaluation of Assay Accuracy

The accuracy of the assay is determined by the standard recovery of each analyte. It is calculated as the percentage of the observed value of a spiked standard of known concentration relative to its expected value; the acceptable recovery is 70–130% of the expected value.

The ability to multiplex is defined when:

- Assays display background MFI ratio <10
- LOD values do not change considerably between groups I and II when tested alone and in combination
- Analyte recovery is between 70 and 130%

Results

For human cytokine analytes, the background MFI values did not change considerably in the 48-plex assay, except for seven group II analytes: CTACK, IL-1α, IL-3, IL-12 (p40), M-CSF, SDF-1α, and TNF-β. These analytes also exhibited high LOD values in the 48-plex assay. To identify which detection antibodies of human cytokine group I contributed to the high background MFI values, single detection antibody cross-reactivity tests were conducted. The group I detection antibodies that cross-reacted nonspecifically to group II capture antibodies are noted in Table 1.

Table 1. Identification of human cytokine group I detection antibodies that cross-react with group II capture antibodies.

Human Cytokine 27-Plex Panel, Group I Detection Antibodies	Human Cytokine 21-Plex Panel, Group II Capture Antibodies																				
	CTACK	GRO-α	HGF	IFN-α2	IL-1α	IL-2Rα	IL-3	IL-12 (p40)	IL-16	IL-18	LIF	MCP-3	M-CSF	MIF	MIG	β-NGF	SCF	SCGF-β	SDF-1α	TNF-β	TRAIL
IL-1β																					
IL-1ra																					
IL-2					•		•	•					•							•	
IL-4																					
IL-5																			•		
IL-6																					
IL-7	•																				
IL-8																			•		
IL-9	•																				
IL-10																					
IL-12 (p70)							•	•					•								
IL-13					•																
IL-15																					
IL-17																					
Basic FGF																					
Eotaxin																					
G-CSF	•																				
GM-CSF																					
IFN-γ																					
IP-10																					
MCP-1 (MCAF)																					
MIP-1α																					
MIP-1β																					
PDGF-BB																					
RANTES																					
TNF-α																					
VEGF	•																				

• Denotes cross-reactivity between group I detection antibodies and group II capture antibodies.

Mouse cytokine assays (groups I and II either individually or in combination) did not show considerable increases in either background MFI or LOD values.

The recovery of all the analytes was 70–130%, within the assay working range, except for CTACK, IL-3, and IL-12 (p40) from the human group II cytokine panel, which resulted in a narrower working range. For mouse group I and II assays, the standard recovery for the 23-plex and the 9-plex panels was comparable to that of the 32-plex format (70–130%), with the exception of these four analytes: IL-1α, GM-CSF, IL-18, and MIP-2. This effect was observed only at the high end of the standard curve and did not impact the sensitivity; therefore, they may be multiplexed with the other analytes.

While cross-reactivity in the majority of the assays was less than 2%, higher cross-reactivity was detected among some analytes, especially those with high sequence similarities. All analytes, with the exception of MIP-1α and MIP-1β, and IL-12 (p40) and IL-12 (p70), demonstrated <2% cross-reactivity for both human and mouse.

Conclusions

The cross-reactivity of group I and group II cytokine panels within each species (human and mouse) was determined.

Based on background MFI and LOD values, all but the following seven assays from group II may be combined with group I assays: CTACK, IL-1α, IL-3, IL-12 (p40), M-CSF, SDF-1α, and TNF-β. The single detection antibody cross-reactivity test identified the specific detection antibodies that caused the high background, with IL-2, IL-12 (p40), and IL-12 (p70) detection antibodies being the major contributors.

Based on the background MFI and LOD values, all mouse groups I and II assays may be combined with minimal impact on assay performance.

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