The Roche New Ultrasensitive SA-PE Improves Sandwich Assay Performance

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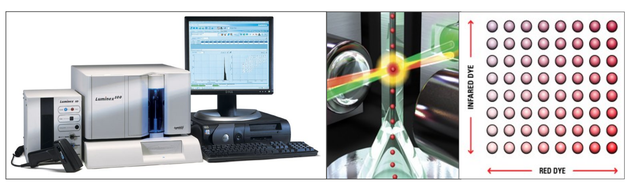
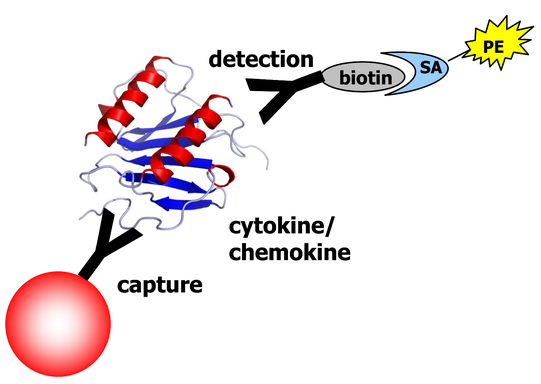
Introduction

Conjugated reporter dyes such as streptavidin-phycoerythrin (SA-PE) strongly affect the performance of multiplexed sandwich immunoassays. High signal intensities combined with very low background signals are a prerequisite for improving limits of detection and quantification. Furthermore, long-term stability and lot-to-lot consistency of such conjugated reporter dyes are important parameters which impact final assay performance, kit convenience, security of supply and cost-effectiveness.
We used multiplexed cytokine assays to evaluate the performance of different streptavidin-phycoerythrin reporter dyes. Limit of quantification and the relative signal intensities were analysed using bead-based sandwich immunoassays. Moreover, the SA-PE performance was assessed using whole blood stimulation assays.

Experimental Setup

For the evaluation of the new Roche SA-PE products, a distinct bead set was coupled with appropriate capture antibodies. Cytokine standards were serially diluted by 4-fold dilution ranging from 10,000 pg/mL to 2.4 pg/mL. After incubation, beads were washed and detection was carried out using biotinylated detection antibodies and SA-PE, read-out was performed using a Luminex 100 system.





Summary

SA-PE products developed by different suppliers greatly influence the assay performance. We compared the new LumiGrade ultrasensitive SA-PE Reagent from Roche to products from other suppliers using our self-developed multiplexed cytokine assays.

- HPLC analysis showed excellent purity of the LumiGrade ultrasensitive SA-PE Reagent.
- Higher signal intensities can be achieved using the new LumiGrade ultrasensitive SA-PE Reagent from Roche in place of SA-PEs from other suppliers.
- The new SA-PE also reveals a high lot-to-lot consistency.
- Low background signals improve signal-to-noise ratio.
- Stimulated whole blood samples were measured.

Results

1. HPLC analysis

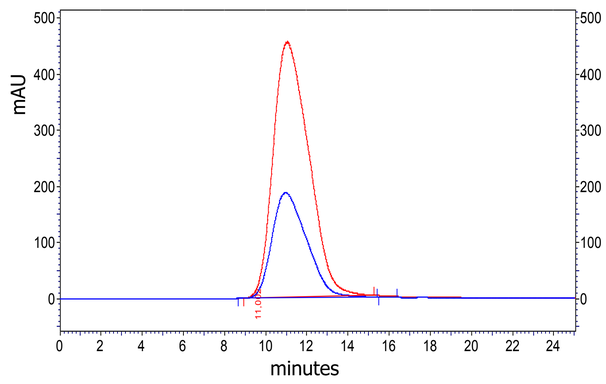
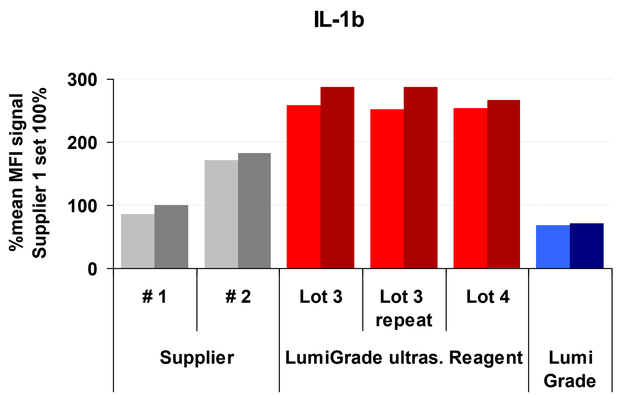


Figure 1: *The LumiGrade ultrasensitive SA-PE Reagent was analysed on a TSK-Gel 6000PWXL column. HPLC analysis showed a uniform product. The amount of free contaminants (SA or PE) was less than 0.3 %. Detection: 280nm (blue), 566nm (red).*

2. Lot-to-lot consistency



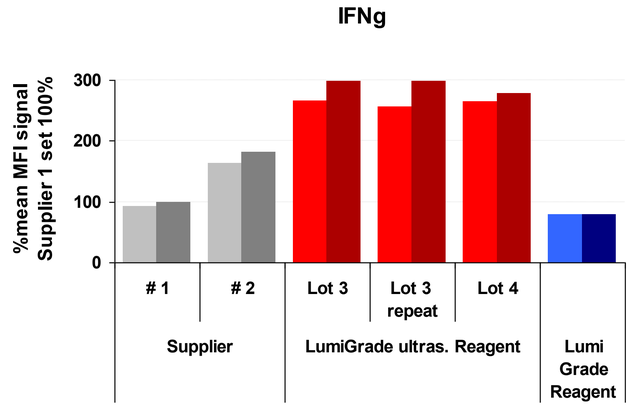
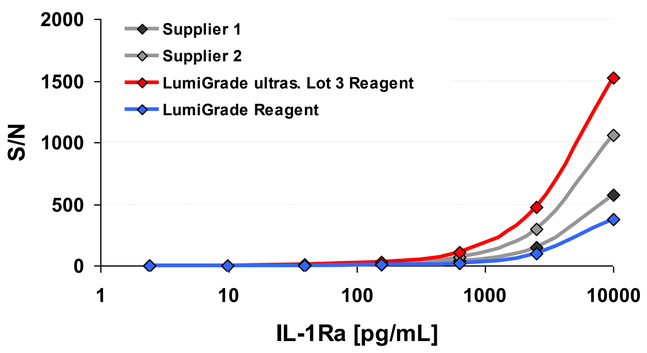


Figure 2: *Lot-to-lot consistency of Roche's new LumiGrade ultrasensitive SA-PE Reagent was tested in a self-developed 5-plex cytokine assay. The new LumiGrade ultrasensitive Reagent showed higher MFI signals than the LumiGrade SA-PE Reagent and than products from other suppliers. A high lot-to-lot consistency was observed.*

The graphs show the % mean MFI signal of two representative standard curves where supplier 1 was set to 100 %. The experiment was performed twice in triplicates.

Experiment 1: light colour; experiment 2: dark colour.

3. Improved S/N in a problematic assay



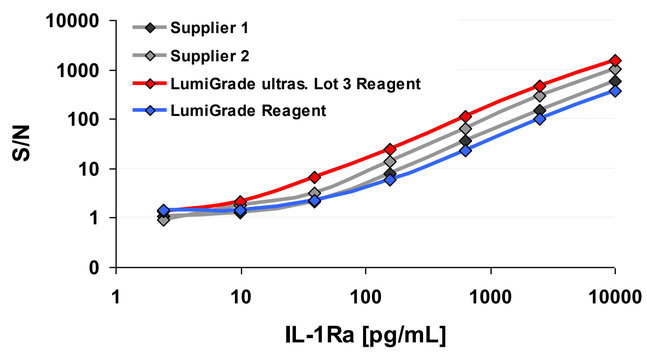


Figure 3: *MFI signals of the IL-1Ra assay were very low compared to other cytokines in both our self-developed and commercially available assays. When the LumiGrade ultrasensitive SA-PE Reagent was used, signal intensity increased, but background values did not (see Table 1).*

Therefore, signal-to-noise ratio improved. The mid- to high-end sensitivities can be seen in the log-linear plot, and the low-end sensitivities in the log-log plot.

Analyte	Exp.	Supplier 1	Supplier 2	LumiGrade ultras. Lot 3 Reagent	LumiGrade ultras. Lot 4 Reagent	LumiGrade
IL-1β	1	6 ± 1	13 ± 2	17 ± 1	28 ± 2	4 ± 1
	2	4 ± 3	3 ± 1	5 ± 1	5 ± 1	3 ± 1
IFNγ	1	2 ± 1	1 ± 1	2 ± 1	3 ± 1	1 ± 1
	2	1 ± 1	1 ± 1	2 ± 1	3 ± 1	2 ± 1
IL-1Ra	1	2 ± 1	2 ± 1	3 ± 1	3 ± 1	3 ± 1

Table 1: *Comparison of Blank MFI values of different SA-PE products (for standard curves see fig. 2 and 3).*

4. Cytokine measurement of stimulated whole blood

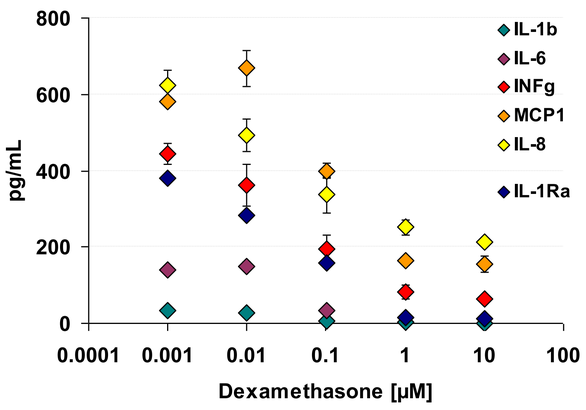


Figure 4: *α-CD3/α-CD28 stimulated whole blood was treated with increasing concentrations of the corticosteroid dexamethasone. Cytokine levels were measured in the supernatant with a 5-plex sandwich immunoassay. IL-1Ra was measured in a separate singleplex assay.*
Detection with Lumi Grade Ultrasensitive SA-PE reagent achieved reliable results. The graphs show the result of one representative experiment done in duplicates.