TECHNICAL NOTE

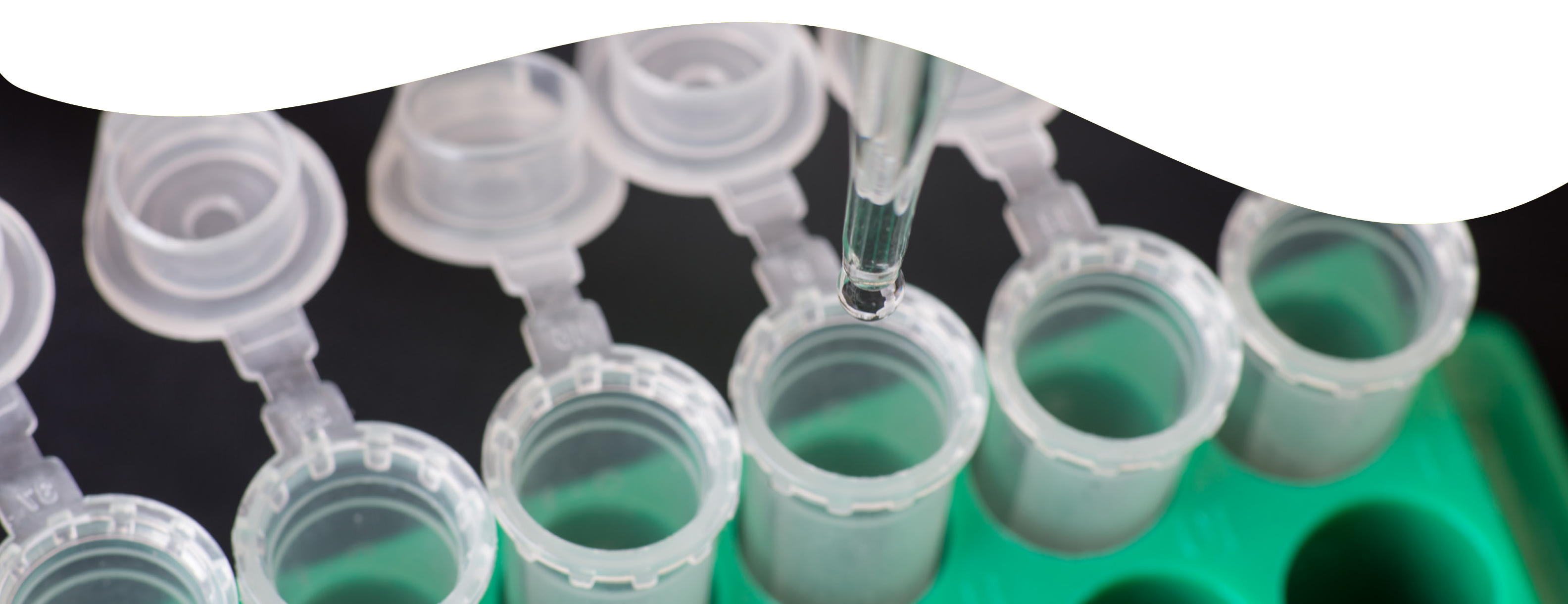
LabChip Protein Express Assay: mitigating effects of laboratory plasticware on assay performance.

Abstract

The use of standard plastic labware has been shown to compromise results of specific bioassays. Here we present data showing positive correlation between the container used for mixing the LabChip™ Protein Express gel/dye and occurrence of baseline dips before peaks. Our study showed that minimizing plastics exposure by mixing gel/dye directly in the spin filter eliminated baseline artifacts across several different lots of reagents.

Introduction

Leachates from disposable plasticware have been studied extensively for contamination of life science reagents[1]. The leaching additives in standard plasticware include coating agents such as silicon, slip agents (e.g. erucamide) and plasticizers used in plastic manufacturing, as well as detergents and dyes that send colors to plastics. Over time, those additives can migrate to the plastic surface and into the solutions contained within the plasticware. Polypropylene is one of the most commonly used plastic for pipette tips and microfuge tubes. Studies show that the suspect contaminants from polypropylene are at very low concentration, 1-10 µM after two months storage of DI water[2], but are significant enough to affect bioassays. Using water rinsed through plastic tubes was found to interfere with enzyme kinetics and the effect was more pronounced when using dimethyl sulfoxide (DMSO)[3].



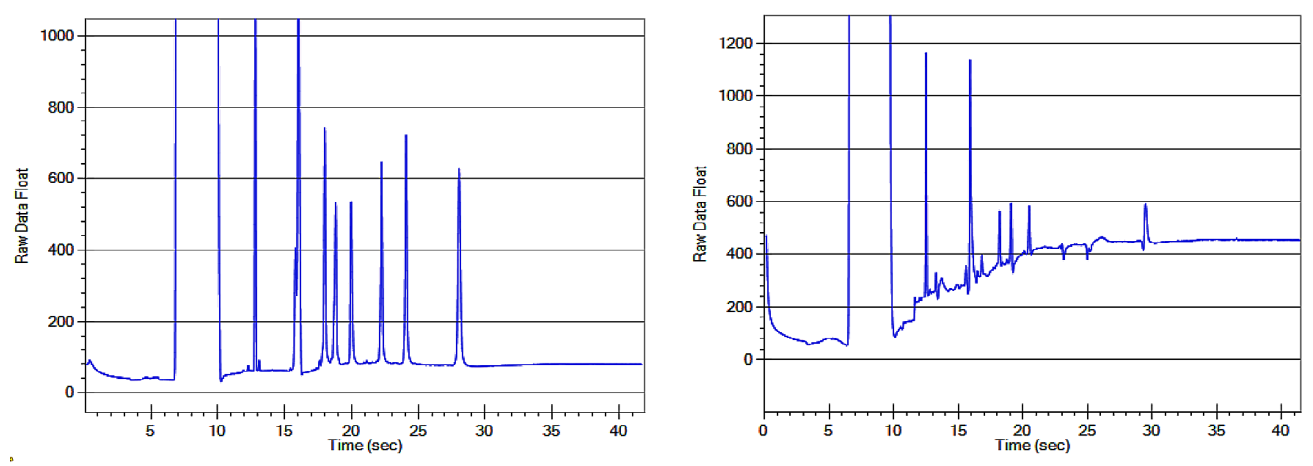


Figure 1: Left: Normal raw data trace of protein ladders. Right: Data showing high baseline (>200 rfu) and baseline dips associated with ladder protein peaks.

The LabChip microfluidic Protein Express assay requires mixing the gel matrix and dye solutions prior to chip preparation. Varying levels of baseline signal and dips were observed in the data when different brands of polypropylene tubes were used. The most significant dips associated with low ladder signal or missing ladder peaks (Figure 1), and impacted the assay's capability to accurately determine sample concentration or to resolve low-level impurities. Here, we take the Corning® Axygen® MCT-175-A MaxyClear Standard Microcentrifuge Tube (1.7mL, assorted colors, PP) as an example to demonstrate the positive correlation between the plastic container used to mix the gel/dye and the presence of dips.

Materials and methods

To maintain reagent consistency, we used bulk (not aliquoted to individual tubes) gel matrix and dye solutions for gel/dye mixing. Gel was stored in a 50 mL Greiner Bio-One polypropylene tube, and dye was stored in a 0.5 mL Nalgene™ amber tube (Thermo Scientific™ 342805-0005). All experiments were performed using the Protein Express 200 assay on the LabChip GXII Touch system in accordance with the user's guide, except that gel and dye volumes were measured separately for mixing.

The method to mix gel/dye in a spin filter is first to transfer 520 µL gel matrix to the top (basket) of a spin filter, then transfer 20 µL dye solution into the gel matrix in the upper part of the spin filter. Cap the spin filter and invert it. Vortex the inverted filter for about 10 seconds until the dye is evenly dispersed. Centrifuge the gel/dye mix and follow the protocol for the remaining steps.

Experimental results

Here we present two sets of experiments to investigate the presence of baseline dips when gel/dye solution is exposed to the Corning® Axygen® tubes.

Gel/dye solution mixed directly in a Corning® Axygen® Tube

520 µL gel matrix and 20 µL dye solution were mixed in either a Corning® Axygen® 1.7 mL tube or a spin filter. The gel/dye solution from the Corning® Axygen® tube was transferred after mixing to a second spin filter and centrifuged down together with the first spin filter containing gel/dye solution. One chip was used successively for two runs with the same ladder solution as a sample.

Figure 2 shows the electropherogram of ladder profiles from gel/dye solution mixed in the Corning® Axygen® tube vs. the spin filter. Elevated baseline and significant baseline dips were observed in data from the run where the chip was prepared using the gel/dye mixed in a Corning® Axygen® tube.

Gel/dye solution pre-mixed and transferred to Corning® Axygen® Tubes

The same chip can produce data both with and without dips, demonstrating that the issue comes from the gel/dye solution. To exclude the gel/dye solution variance from preparations, we made a bulk gel/dye solution (> 5 mL) in a 50 mL Greiner Bio-One polypropylene tube. 600 µL of pre-mixed gel/dye solution was transferred to two Corning® Axygen® tubes each, and the tubes were put on a slow rotator for 2 hours to let the solution contact the plastics. 300 µL of gel/dye solution was then transferred individually from the Greiner Bio-One tube and the two Corning® Axygen® tubes to three spin filters. The same ladder sample was used for runs with each of the gel/dye mixes.

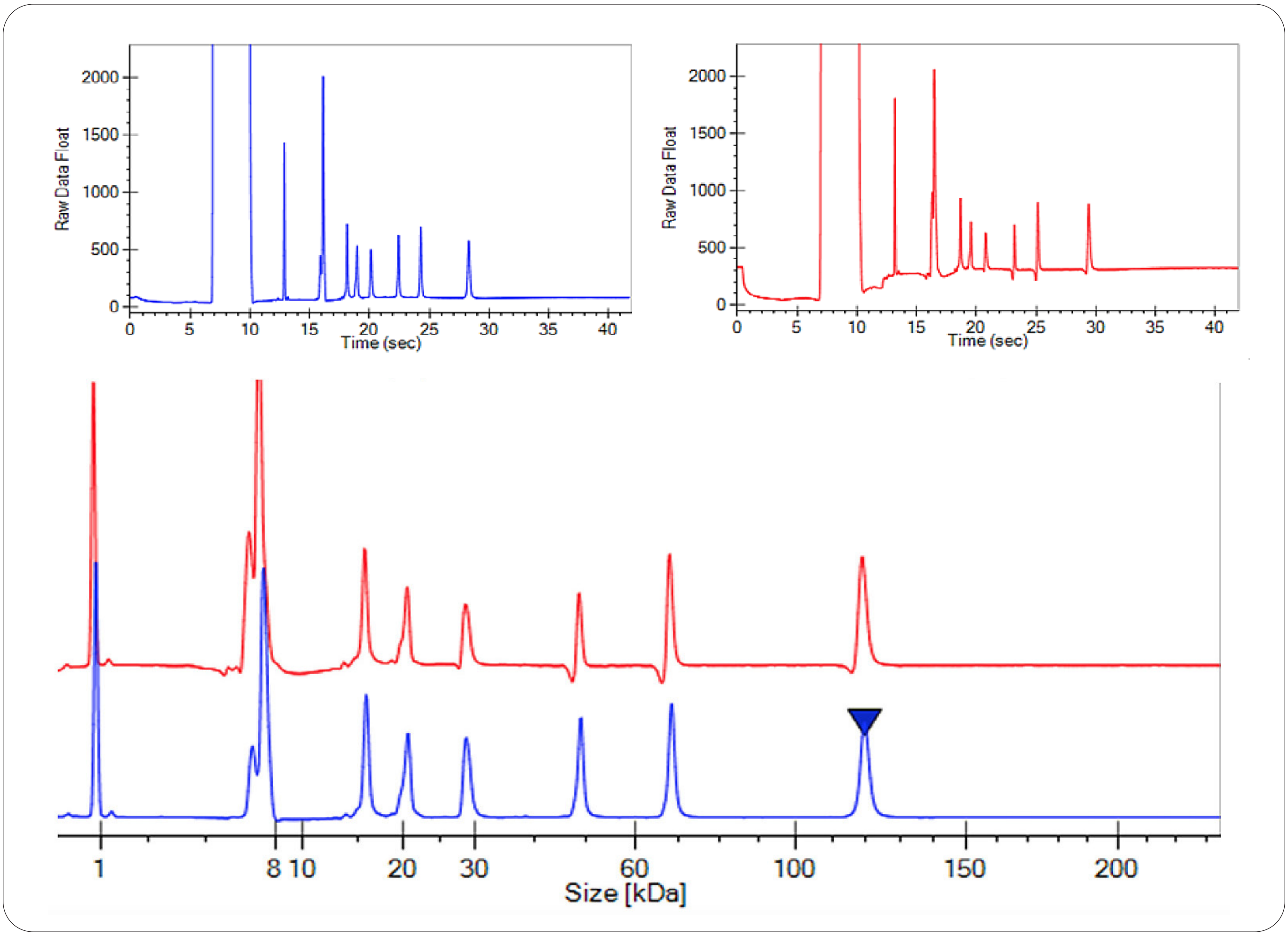


Figure 2: Top left: raw data trace showing typical ladder profile from the spin filter mix. Top right: raw data trace showing high baseline and dips from the Corning® Axygen® tube mix. Bottom: analyzed data showing the ladder profile from gel/dye mixed in a Corning® Axygen® tube (red trace) and a spin filter (blue trace).

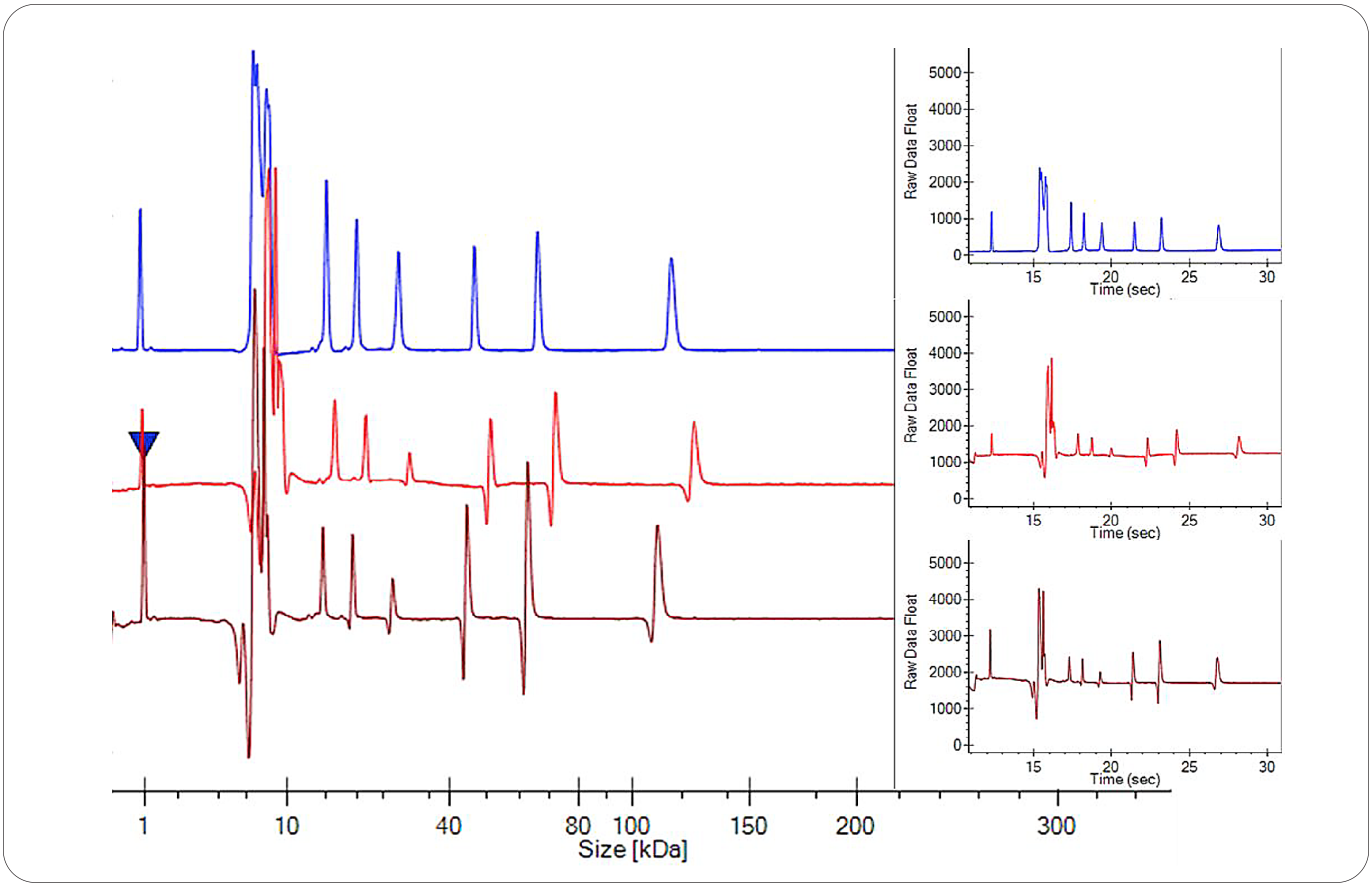


Figure 3: Analyzed data showing ladder profiles from using gel/dye solution incubated in the Greiner Bio-One tube (blue trace) and the Corning® Axygen® tubes (red and brown traces). Inset top: raw data trace showing typical ladder profile from the Greiner Bio-One tube. Inset middle and bottom: raw data traces showing high baseline and dips from the Corning® Axygen® tubes.

Figure 3 shows the electropherogram of ladder profiles using the gel/dye mix from the Corning® Axygen® tubes and the Greiner Bio-One tube. Elevated baseline and severe dips were observed from both gel/dye mixes that were incubated in Corning® Axygen® tubes.

The table below summarizes the data from Figure 2 and 3 for quantitative comparisons.

Table 1: Quantitative summary of baseline artifacts observed with gel/dye mixes made in different plastic containers.

	Plastic container used	Dips height*	70 kDa Peak height	Dips ratio**	Baseline	Chip ID
Figure 2	Corning® Axygen® tube mix	100	588.72	17%	350	L393P-0563N-02
	Spin filter mix	0	610.32	0%	90	
Figure 3	Corning® Axygen® tube_1	500	1139.1	43.9%	1700	L454P-0563N-01
	Corning® Axygen® tube_2	300	684.5	43.8%	1200	L393P-0563N-04
	Greiner Bio-One tube	0	895.34	0%	130	L454P-0563N-01

*The lowest signal immediately before the 70 kDa ladder peak was used to quantify the baseline dips in rfu.

**Dips ratio indicates the magnitude of the baseline dip relative to the height of the 70 kDa ladder peak.

Summary

The performance of the Protein Express 200 assay on the LabChip microfluidics platform can be affected by contaminants from plastic containers. Our testing results strongly suggest that gel/dye mix leaches impurities from Corning® Axygen® polypropylene tubes. Some levels of contamination can be eliminated by filtering so that data is not affected when the gel/dye contacts only certain brands of containers. Specific types or high levels of contaminants from polypropylene labware will affect data even after filtering. These observations have led us to recommend a protocol in which the gel and dye are mixed directly in the top of the spin filter, minimizing exposure of the gel/dye to plastics during preparation.

References

1. McDonald G. R, Kozuska L. J and Holt A. Bioactive leachatesfrom lab plastics. G.I.T. Laboratory Journal 2009, 9-10.
2. Xu Zh. et al. Quick detection of contaminants leaching frompolypropylene centrifuge tubes with surface-enhanced Ramanspectroscopy and ultraviolet absorption spectroscopy. J. RamanSpectrosc. 2011, 42, 1939-1944.
3. McDonald G. R et al. Bioactive contaminants leach fromdisposable laboratory plasticware. Science 2008, 322, 917-917.