# **Electronic supplementary information (ESI)**

# High-throughput fluorescence polarization immunoassay by using a portable fluorescence polarization imaging analyzer

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### Fabrication of the mixing microdevice

Mixing of deoxynivalenol (DON) sample solution containing tracer with antibody solution was done with the microdevice, and then, the mixture was directly measured by the FP analyzer. The mixing microdevice was fabricated using the standard soft lithography technique with some modifications.<sup>1,2</sup> Negative photoresist SU-8 3050 (Nippon Kayaku Co., Ltd., Tokyo, Japan) was spin-coated onto two different silicon wafers (Sumco Co., Tokyo, Japan) at 2700 rpm for a 50 µm thick control layer (CL) mold, and at 1300 rpm for a 100 µm thick fluid layer (FL) mold, respectively. After backing at 95 °C for 17 min for CL or 45 min for FL, the wafers were exposed to UV light which passed through photomasks designed by a mask aligner (M-1S, Mikasa Co., Ltd., Tokyo, Japan) to complete fabrication of the molds. For fabrication of the microdevice, the molds were coated with trichloro(1H, 1H, 2H, 2H-perfluorooctyl) silane. The CL mold was spin-coated with PDMS (Sylgard 184 Silicone Elastomer Kit, Dow Corning Toray Co., Ltd., Tokyo, Japan) to fabricate the CL with a thickness of 70 µm. The FL mold also was spin-coated with PDMS to fabricate the FL with a thickness of 120 µm. The top of the CL was bonded to a 50 µm thick cyclo olefin polymer film (ZeonorFilm ZF14-050, Zeon Co., Tokyo, Japan) using an oxygen plasma generation apparatus (CUTE-1MP/R, Femto Science, Gwangju, Korea). After peeling off the mold, the CL, which the microchannel design had been transferred to, was aligned to the FL which the microchannel design had been transferred to. After baking for at least 4 h at 80 °C, the aligned CL and FL were pasted onto a glass slide. Because of the fabrication strategy, the microdevice had no black silicon rubber and was a clear PDMS microdevice.

#### Orientation of solution mixing on the microdevice

Mixing using the microdevice was done according to instructions of the references.<sup>1, 2</sup> Fig. S1(A) shows a schematic illustration of the mixing microdevice filled with antibody solution and analyte solution which included tracer. A schematic illustration of the experimental procedure is shown in Figs. S1(B) and S1(C). Briefly, an antibody solution and a mixture of the analyte and tracer solutions were introduced into separate microchambers of the FL. When the partition valve was pulled up by decompressing the microchannel of the CL, these solutions were mixed. The sample mixture was directly measured by the developed FP analyzer. The volume of sample mixture was calculated to be 4 nL.



**Fig. S1** Cross-sectional illustrations of the microdevice. (A) The antibody solution and the mixed solution of analyte and tracer were introduced into the microchamber of the FL. (B) The antibody solution and the mixture solution of analyte and tracer were mixed by pulling up the partition valve. (C) Contents of these microchambers were directly measured by the FP analyzer.

#### FP measurement for on-device mixed deoxynivalenol (DON) samples

The mixing of DON sample solution containing tracer with antibody solution was done using the microdevice, and then, the mixture was directly measured by the developed portable FP analyzer. The FPIA measurements of DON concentrations (2.4, 19.2, and 153.8 ng/mL) were conducted. Fig. S2 shows a typical DC image obtained by FP imaging for DON sample and a schematic illustration explaining the image. The antibody solution was introduced via the upper channel and the DON solution containing tracer was introduced via the lower channel. Because of the transparency of the PDMS microdevice, the background noise is high; however, the occurrence of mixing could be observed. Fig. S3 shows sets of standard curves of the *P* values against DON concentrations obtained by the portable FP analyzer and a conventional apparatus (FP-715, JASCO Co., Tokyo, Japan). The FP relaxation was observed with increasing DON concentration. The results showed that the FP analyzer has a great potential for obtaining further high-throughput FPIA by integrating the mixing steps for the FPIA reactions.



**Fig. S2** Typical DC image for DON sample obtained by the developed FP analyzer and a schematic illustration explaining it. The antibody solution was introduced via the upper channel and the DON solution containing tracer was introduced via the lower channel.



**Fig. S3** Standard curves of FP against DON concentrations obtained using the portable FP analyzer and a conventional apparatus. Mixing of antibody solution with the DON solution which contained tracer was done on the microdevice. The measurements were performed in triplicate.

## References

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