Supplementary Information (SI) for Analyst.
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Sensitivity-Improved Blocking Agent-Free Fluorescence Polarization Assay Through Surface Modification Using Polyethylene Glycol

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Table of Contents.

1. The results of the optimization of PEG-PDMS fabrication process

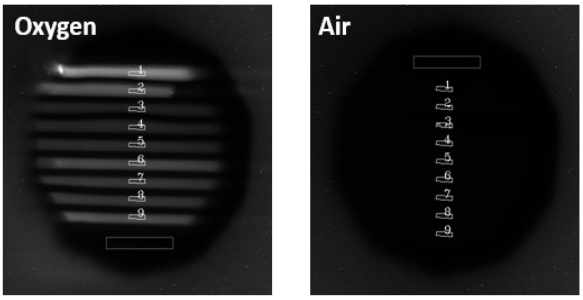

Figure S1. Effect of plasma treatment process gas on the inhibitory adsorption effect of PEG-PDMS. Plasma treatment was performed using oxygen and air as process gases respectively. The fabricated PEG-PDMS device channels were immersed in 10^{-7}, $10^{-7.5}$, and 10^{-8} M FITC-BSA for 1.5 hours and it was washed with PBS. Then, fluorescence images were taken using a portable analyzer.

Table S1. Summary of bonding stability of device with difference heating time

Heating time(hour)		2	4	7	10	15	19
Stability	Chip1	△	○	△	○	◎	◎
	Chip2	×	○	○	○	◎	◎
	Chip3	△	△	○	◎	◎	◎

×:Unstable, △: Part of the PEG-PDMS is unstable,○: Stable,◎:Most Stable

2. The results of the evaluation of the inhibitory adsorption effect using FITC- lysozyme

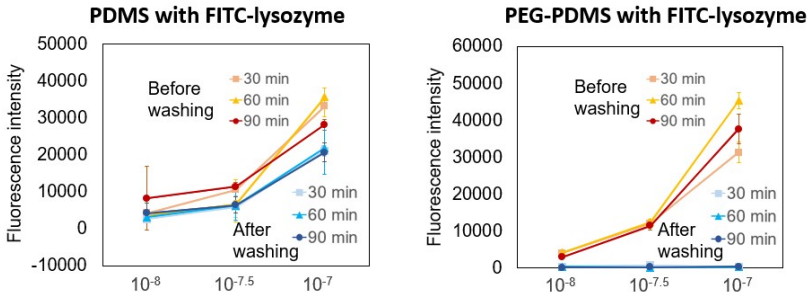

Fig S2. Fluorescence intensity plot of FITC- lysozyme in PEG-PDMS devices.

3. The effect of blocking agents on the sensitivity of FP assay at low concentrations

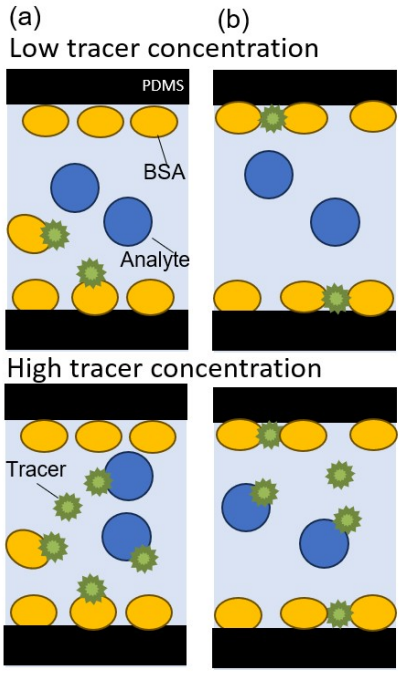

Fig S3. The possible interference on FPIA caused by PDMS channel with blocking agents. at low tracer concentrations. (a) Non-specific binding of tracer to blocking agent. (b) Insufficient surface coverage. Although the amount of adsorbed tracer and analyte is not significant in the high concentration condition, it is significant in low concentration condition.

4. Nonspecific adsorption between tracer and BSA

Fig S4a and S4b shows that, after washing the channel, the fluorescence intensity of both 1 nM and 0.1 nM tracers were consistent at different BSA concentrations, indicating that the tracer did not adsorb to the channel surface in all the BSA concentration conditions. On the other hand, before washing, the trends of fluorescence intensities are different between 1 nM and 0.1 nM tracers. In 1 nM tracer condition, both fluorescence intensity (Fig S4a) and polarization (*P*) (Fig S4c) were constant on the BSA concentration as we expected. However, in 0.1 nM tracer condition, both fluorescence intensity and *P* increased with the BSA concentration (Fig S4b and S4c). These results indicate that the intensity of BSA autofluorescence is comparable fluorescence intensity of 0.1 nM tracer in this wavelength condition. As a result, the increase of *P* with BSA concentration (Fig S4c) cannot be attributed to the nonspecific binding because BSA autofluorescence can increase *P* as well as nonspecific binding.

Next, in order to exclude the influence of autofluorescence, the excitation/emission wavelengths were changed from 490/525 nm to 650/671 nm. The same experiment using Alexa Fluor® 647-Fab was repeated, instead of Alexa Fluor® 488-Fab, as the tracer. As shown in Fig S4d and S4e, the fluorescence intensities before washing were constant on BSA concentration in both 1 nM and 0.1 nM tracer condition, resulting this condition reduced the autofluorescence effect. However, *P* still increased in 0.1 nM tracer condition (Fig S4f). This result indicates that nonspecific binding between BSA and tracer actually increases *P* in 0.1 nM tracer condition.

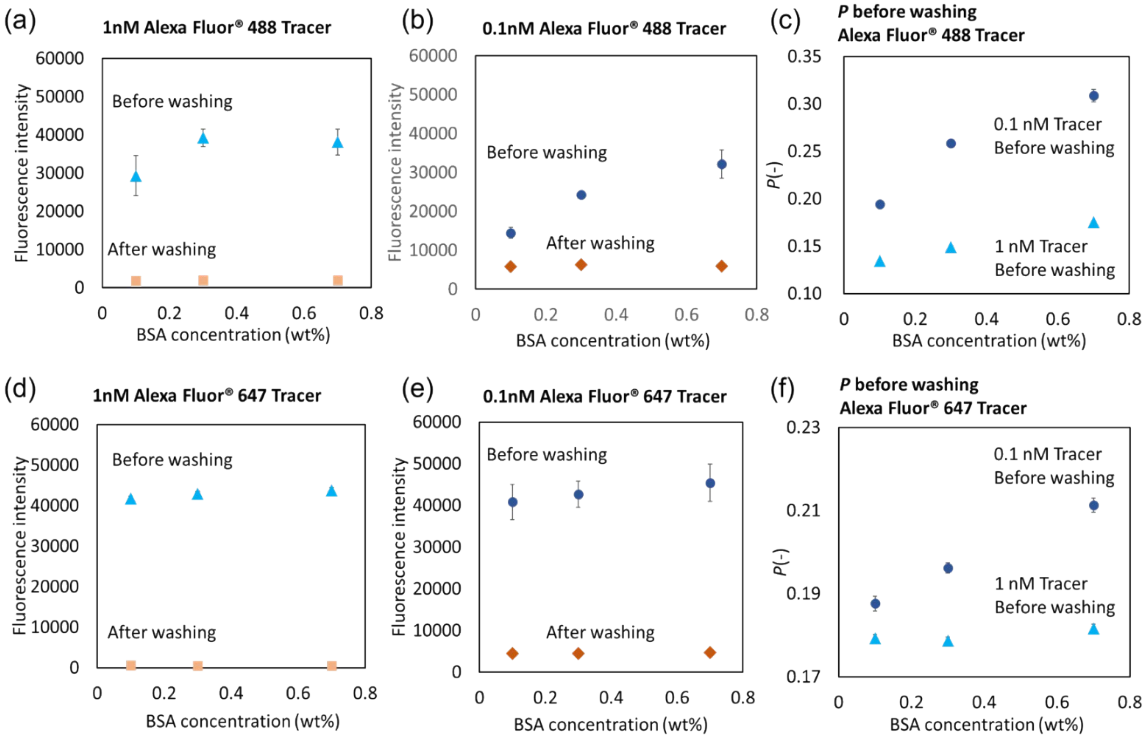

Fig S4. Nonspecific adsorption between tracer and BSA. (a) & (b) Fluorescence intensity (FI) before and after washing of 1 nM (a) and 0.1 nM (b) Alexa Fluor® 488-Fab at different BSA concentrations. (c) *P* of Alexa Fluor® 488 tracer at different BSA concentrations before washing. (d) & (e) FI before and after washing of 1 nM (d) and 0.1 nM (e) Alexa Fluor® 647-Fab at different BSA concentrations. (f) *P* of Alexa Fluor® 647-Fab at different BSA concentrations before washing