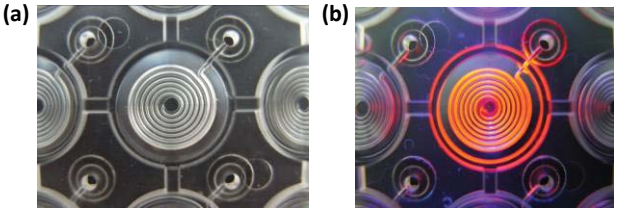
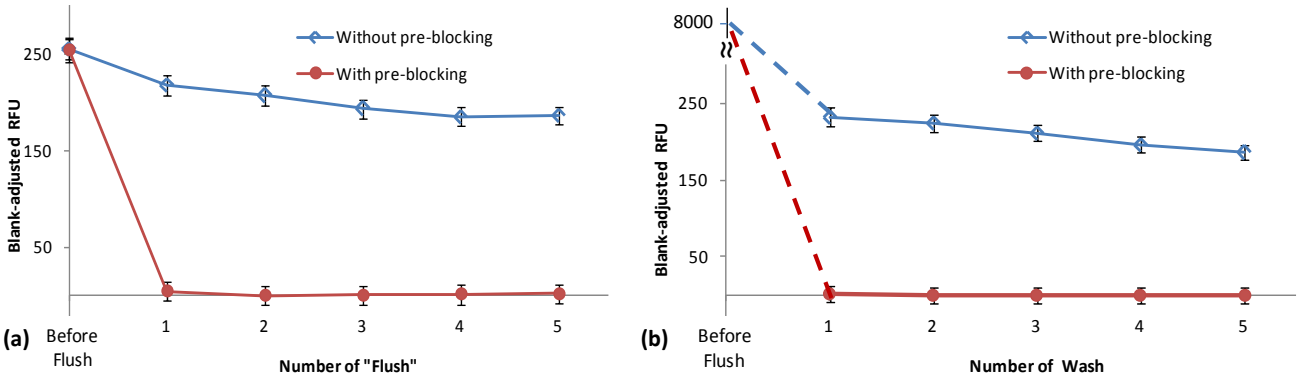
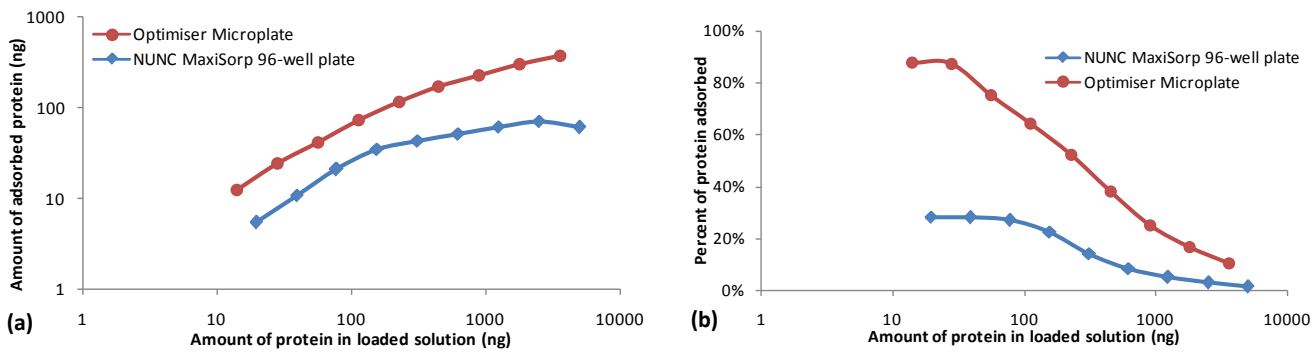
Supplementary Figures and Legends



Supplementary Figure 1. Image of the light emission from the microchannel filled with fluorescence dye: (a) without emission and (b) with emission. Five microliter of high concentration fluorescence dye was filled into the microchannel of one well in Optimiser. Photographs were taken with and without emission light.

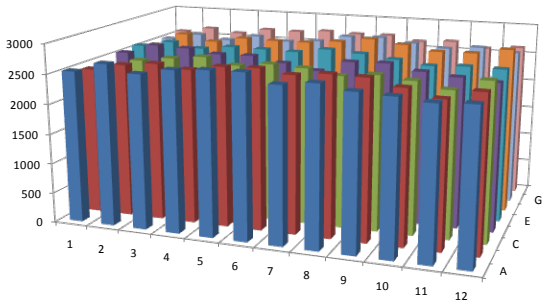


Supplementary Figure 2. a) Efficiency of "flush" in Optimiser plate, and b) Efficiency of conventional wash in NUNC 96-well plate. To demonstrate the efficacy of the flush mechanism, 5 µL blocking buffer was loaded into Optimiser microplate. After 10 minutes incubation, 5 µL of 16 µg/ml FITC labelled IgG was loaded into the Optimiser plate and incubated for 10 minutes. Then the plate was loaded with 10 µL of washing buffer into each well to perform a "flush". After all wells empty, the light intensity of each well was measured by the plate reader. The "flush" procedure is repeated 4 more times with read after each "flush". The results shows that with two "flushes" the signal reaches a stable background level (zero). Note that 2 "flushes" imply 2x volume (20 µL) loaded in a SINGLE step. Similar experiment has been conducted with no blocking buffer loaded in beginning. The results confirm that the protein is firmly attached to the surface and not displaced by the "flush" action. Consequently the drop in signal to ~ 0 for the case where the surface is blocked confirms that all the "unbound" FITC-IgG were effectively flushed out. A similar experiment also performed in NUNC MaxiSorp 96-well plate by washing with 300 µL washing buffer each time shows similar trends confirming that the flush action on Optimiser is equally effective as traditional wash step.



Supplementary Figure 3. The characteristics of IgG adsorption on Optimiser microplate and NUNC MaxiSorp 96-well plate. (a) Adsorption behaviour of IgG protein in a log-log plot. (b) Percent of IgG adsorbed comparing to added. A comparative experiment using Optimiser microplate and NUNC MaxiSorp high-binding 96-well plate was conducted to study IgG adsorption. FITC labelled IgG solutions with various concentrations have been prepared. For Optimiser, 4.5 µL of each FITC-IgG solution was loaded into corresponding well and incubated for 10 minutes. For NUNC plate, 100 µL of each FITC-IgG solution was loaded into corresponding well and incubate for 1.5 hours. After incubation, the Optimiser was "flushed" with 2 times 30 µL of wash buffer. And the NUNC plate

was washed with 3 times 300 µL of wash buffer. The amount of IgG added was calculated based on concentration and solution volume (4.5 µL for Optimiser and 100 µL for NUNC plate). The amount of bound protein was calculated based on the measured light intensity.



Supplementary Figure 4. Assay uniformity of the 96 wells on Optimiser microplate. 20 ng/mL mouse IgG was incubated for 10 minutes in each microfluidic chamber of the Optimiser plate, then the surface was blocked and "flushed" to remove unbound materials. HRP-conjugated anti-mouse IgG was added and after flushing away excess the bound fraction was detected using chemifluorescence substrate read by a fluorescence reader. The assay CV cross the plate is less than 4%.