Supplementary Information

Antibody-Free Microfluidic Paper-Based Analytical Device for the Determination of Tear Fluid Lactoferrin by Fluorescence Sensitization of Tb^{3+}

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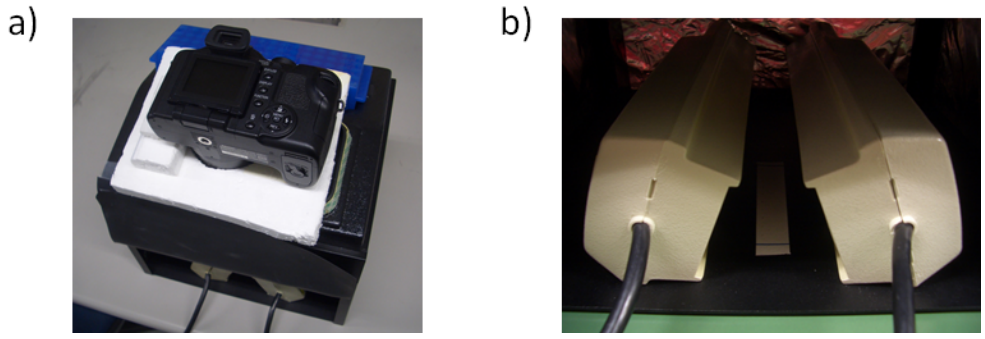

Figure S1: a) Photograph of the experimental setup used for obtaining images of fluorescence emitted from the μPAD. The μPAD is placed between two UV hand lamps (λ_{ex} = 254 nm) inside a darkened cabinet. The fluorescence signal is captured through the window on the topside of the cabinet by a standard digital camera with a 520 nm longpass filter attached to the lens. Shutter speed and aperture of the camera have been set to fixed values in the manual mode, to avoid uncontrolled automatic camera adjustments. b) The inside of the cabinet is covered with aluminum foil and ambient light is completely blocked by a black-out curtain.

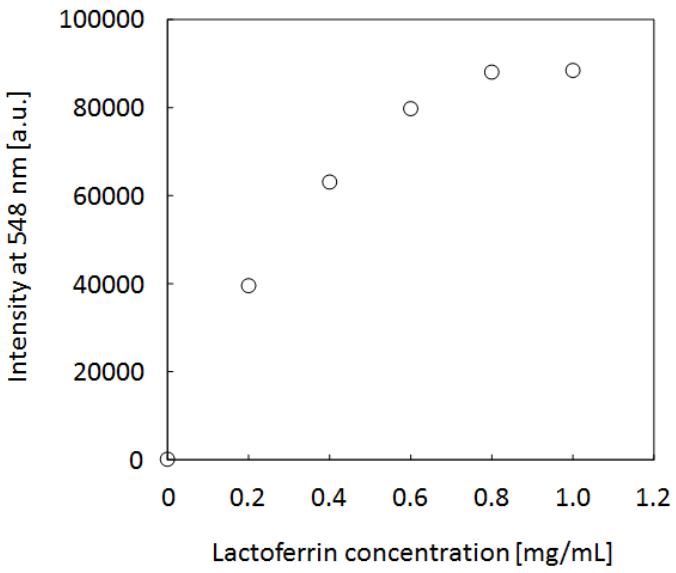

Figure S2: Lactoferrin concentration dependent fluorescence emission of Tb^{3+} (100 μM) in solution (50 mM HEPES, 3.75 mM $NaHCO_3$, pH 7.4); the corresponding spectra are shown in Fig. 3 of the main text.

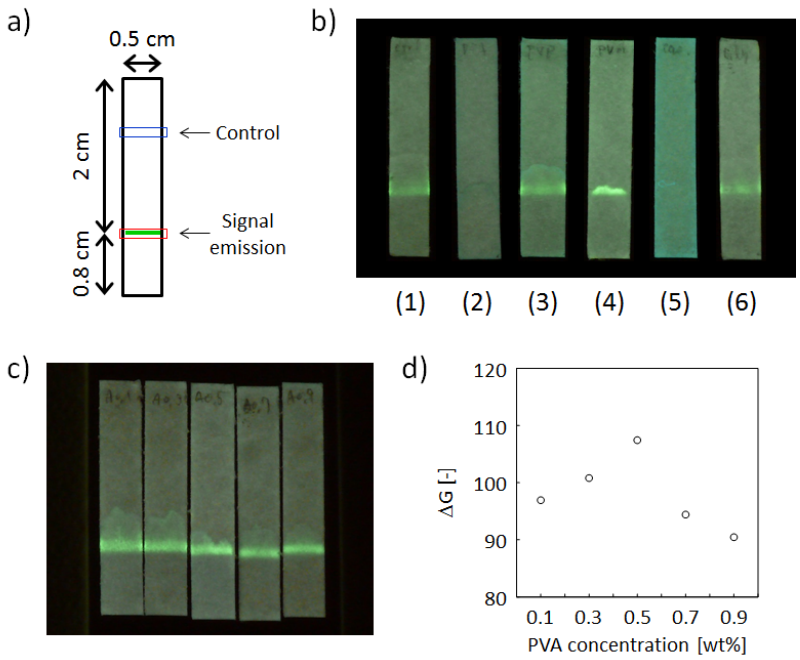

Figure S3: Optimization study of the surface treatment reagent. Performance was evaluated as follows: (i) 4 layers of $TbCl_3$ solution (1 mM in pH 7.4 HEPES buffer) printed in a linear fashion onto No. 5C filter paper; (ii) cut into 0.5 x 2.8 cm^2 strips; (iii) soaked in surface treatment solution for 5 min and dried for 20 min at 37 ˚C; (iv) elution of lactoferrin by placing the bottom of the strip into a lactoferrin solution (2 mg/mL in pH 7.4 HEPES buffer). a) Dimensions of the strip. The position of printed $TbCl_3$ is indicated as a green line. b) Images of strips under UV light (λ_{ex} = 254 nm) after surface treatment with various substances: (1) control, (2) 0.5 wt% bovine serum albumin, (3) 0.5 wt% poly(vinyl pyrrolidone), (4) 0.5 wt% poly(vinyl alcohol), (5) 0.5 wt% casein, (6) 1 wt% glycerol. c) Images of strips under UV light after surface treatment with various concentration of poly(vinyl alcohol): 0.1, 0.3, 0.5, 0.7, 0.9 wt% (from left to right. All reagents were prepared in pH 7.4 HEPES buffer (50 mM). d) Optimization study of PVA concentration: ΔG indicates the difference of the G value between the red (signal) and blue (control) areas shown in Fig. S3(a).

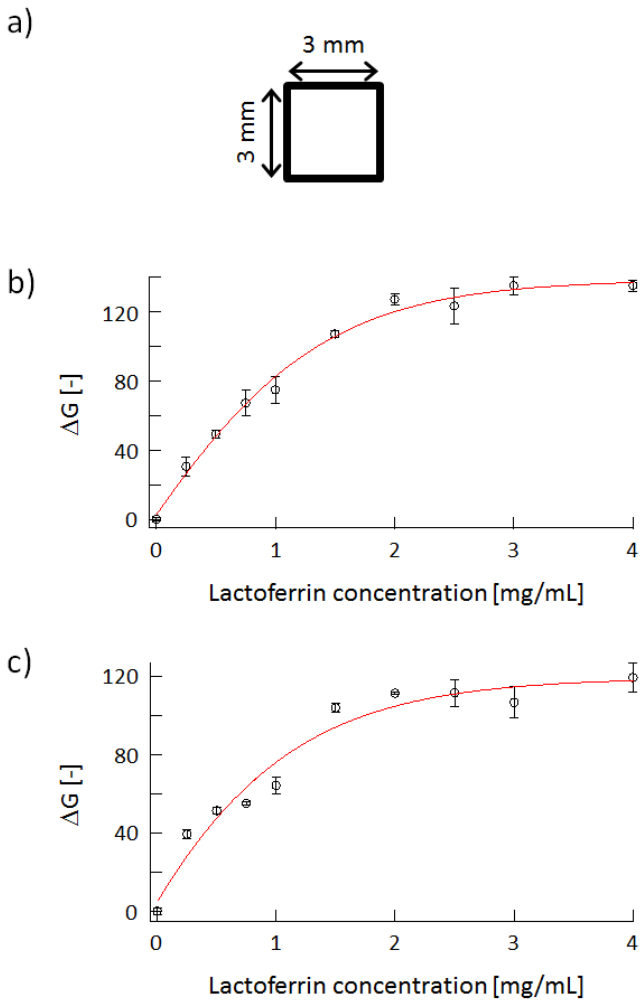

Figure S4: a) Dimensions of a spot test for evaluating the advantage of separated sampling and sensing areas. b) Lactoferrin calibration curve in the case of both $TbCl_3$ and $NaHCO_3$ printed onto the spot. c) Lactoferrin calibration curve with only $TbCl_3$ printed onto the spot. Printing layers of $TbCl_3$ and $NaHCO_3$ were 8 and 12, respectively. Blocking treatment was performed as described in the experimental section. The sample volume applied was 2.5 μL. The markers and error bars reflect the average and standard deviations of three measurements.

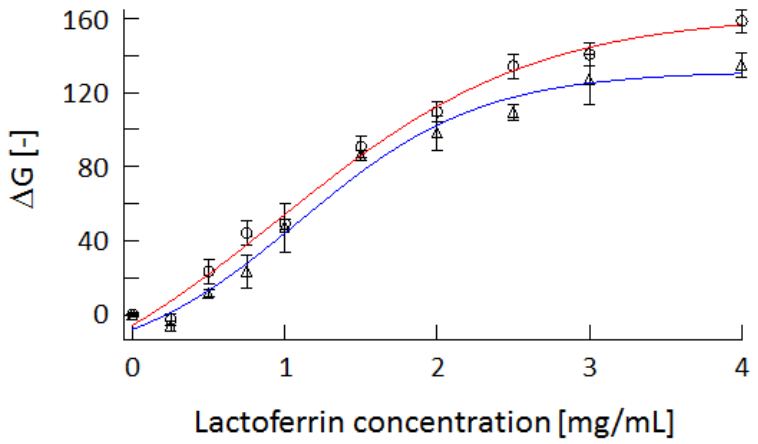

Figure S5: Experimental evaluation of the influence of $NaHCO_3$ on the lactoferrin calibration curve. Data was obtained with μPADs fabricated with (red curve) and without $NaHCO_3$ (blue curve) printed onto the sampling area. An enhanced response is observed in the presence of $NaHCO_3$ in the sampling area due to promoted complexation of lactoferrin and Tb^{3+}. Printing layers of $TbCl_3$ and $NaHCO_3$ were 8 and 12, respectively. The sample volume applied was 2.5 μL. The markers and error bars reflect the average and standard deviations of three measurements. Every single measurement has been performed on a separate *μ*PAD.

Table S1: Material cost estimation

Item	Cost per single μPAD[a]
Filter paper	$0.0071
Copy paper	$0.0003
UV ink	$0.0012
Assay reagents	$0.0045
Total	**$0.0131**

[a] 72 μPADs are printed per sheet.

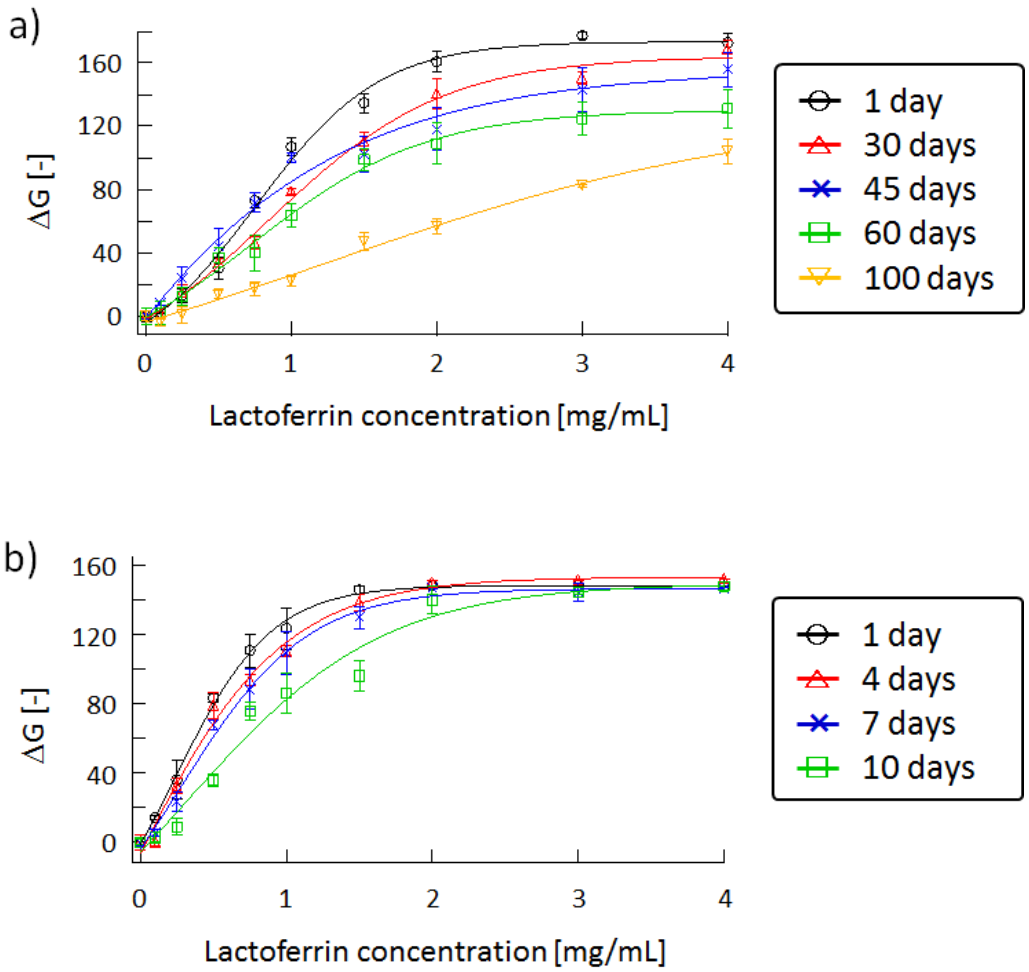

Figure S6: Investigation of the storage stability of *μ*PADs. Calibration curves for lactoferrin were obtained by applying 3 μL samples onto μPADs stored in the dark at a) room temperature (25 ˚C); and b) in a climate control chamber at 35 ˚C and 50% relative humidity. The markers and error bars reflect the average and standard deviations of three measurements.

Table S2: Limits of detection (LOD; 3σ) and limits of quantification (LOQ; 10σ) for lactoferrin measured with μPADs stored in the dark at a) room temperature (25 ˚C); and b) in a climate control chamber at 35 ˚C and 50% relative humidity.

a)

Storage period [days]	LOD [mg/mL]	LOQ [mg/mL]
1	0.20	0.46
30	0.12	0.28
45	0.06	0.16
60	0.30	0.84
100	0.49	1.20

b)

Storage period [days]	LOD [mg/mL]	LOQ [mg/mL]
1	0.11	0.28
4	0.12	0.32
7	0.11	0.20
10	0.12	0.24

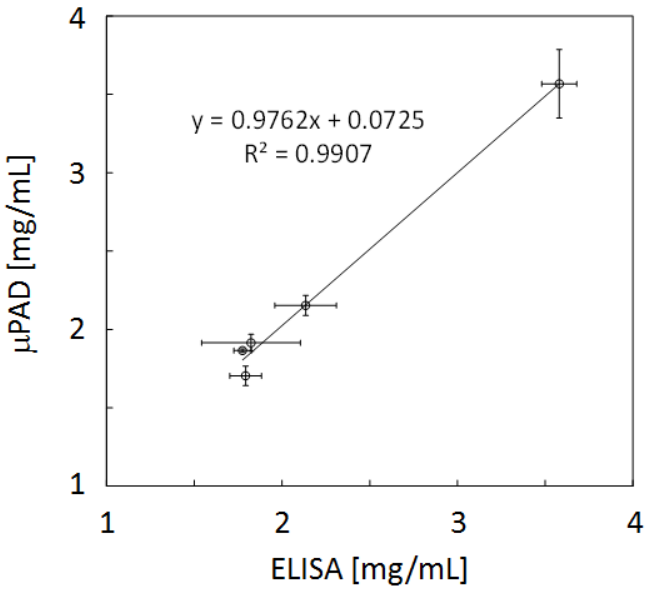

Figure S7: Correlation between lactoferrin concentrations in human tear fluid measured by the ELISA and the µPAD methods; the markers and error bars reflect the average and standard deviations of four (ELISA) and five (µPAD) measurements, respectively.