Supplementary Information for

Printed low-cost microfluidic analytical devices based on a transparent substrate

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The amount of nitroso-PSAP printed onto µTFADs

10 mM nitroso-PSAP was deposited in the form of a circle of 5.0 mm diameter using the black ink cartridge of the Canon iP2700 inkjet printer. Based on the calibrated printing volume (refer to reference 36 of the main text), a single printing cycle results in the deposition of approximately 1400 nL/cm^2, corresponding to an amount of approximately 2.75 nmol of nitroso-PSAP in a 5.0 mm diameter spot. This colorimetric indicator is known to form 1:4 complexes with Fe^{2+}. The formation of 1:4 complexes with 50 µL of a 200 µM Fe^{2+}-sample (10 nmol Fe^{2+}) requires 40 nmol of nitroso-PSAP printed onto a single µTFAD. This is achieved by printing 15 cycles of the reagent ink.

Microplate well test to identify assay component mixing incompatibility

To identify pre-mixing incompatibility of the three assay components (ascorbic acid, phosphate buffer, nitroso-PSAP), mixtures of the following two components were initially added to microplate wells:

(i) ascorbic acid, nitroso-PSAP

(ii) pH 6.8 phosphate buffer, nitroso-PSAP

(iii) ascorbic acid, pH 6.8 phosphate buffer

After overnight storage under dark and refrigerated condition (4°C), the residual relevant component and the Fe^{2+} sample were added:

(i) pH 6.8 phosphate buffer, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$

(ii) ascorbic acid, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$

(iii) nitroso-PSAP, $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$

200 μL of the final resulting mixture composed of ascorbic acid (4 mM), pH 6.8 phosphate buffer (10 mM), nitroso-PSAP (1 mM), and Fe^{2+} (0, 1, 2, 4, 6, 10 μM) was transferred to a separate microplate well and absorbance spectra were immediately acquired by the microplate reader. The results are shown in Figs. S5a, 5b, 5c for conditions (i), (ii), (iii), respectively.

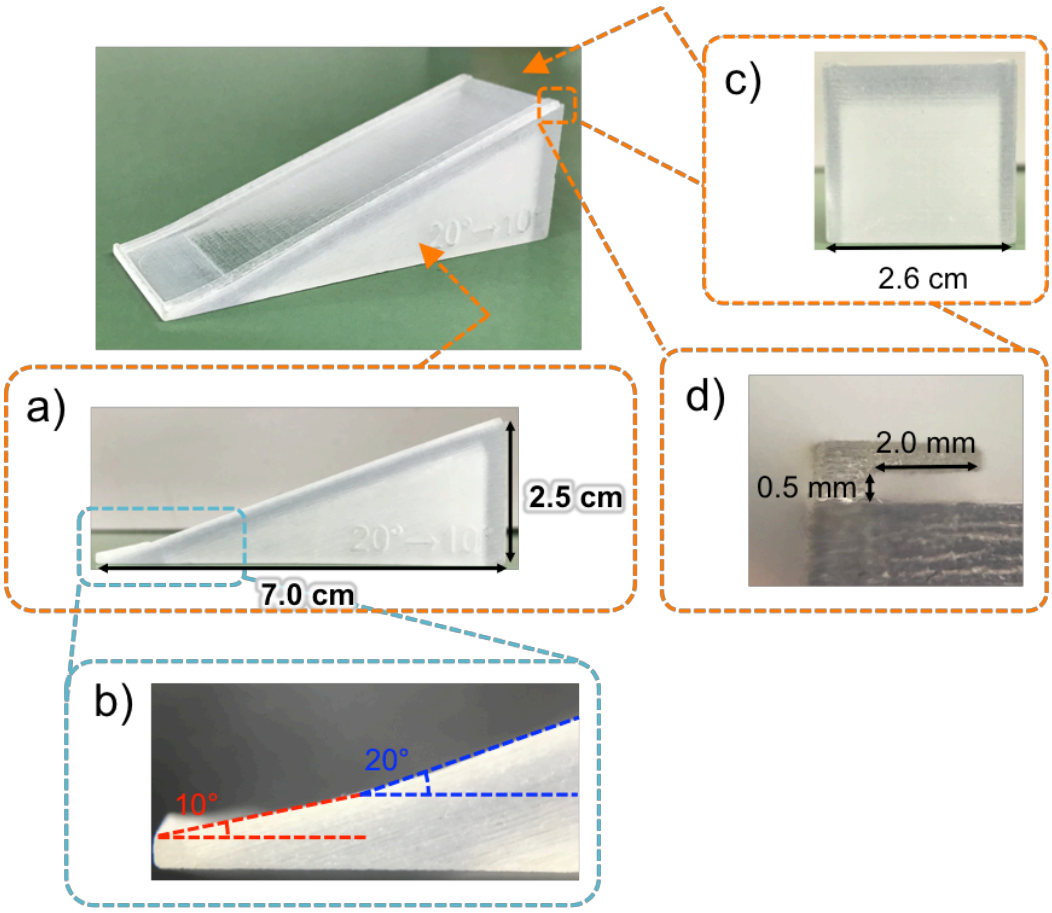

Figure S1: Schematic outline of 3D-printed inclination stand: a) a side view; b) a 10-degree slope and 20-degree slope at the bottom part; c) back face; d) slot on the edge for device fixation.

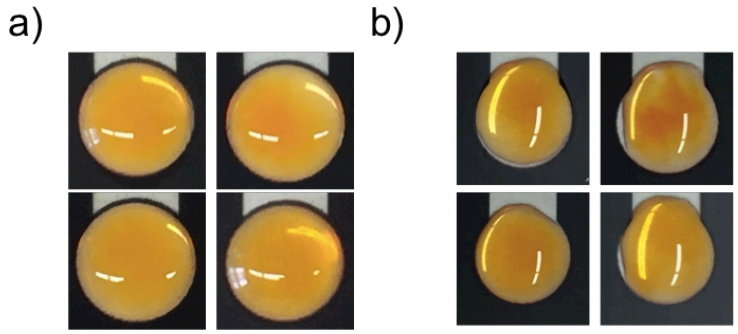

Figure S2: Optimization of the sensing area. Photographs of the sample droplet in the sensing area a) surrounded by a circular wax barrier (0.05 mm width); b) without the circular wax barrier. Each result was obtained from four independent experiments. The sample droplet only formed reproducible circular shapes in the presence of the wax barrier in front of the sensing area.

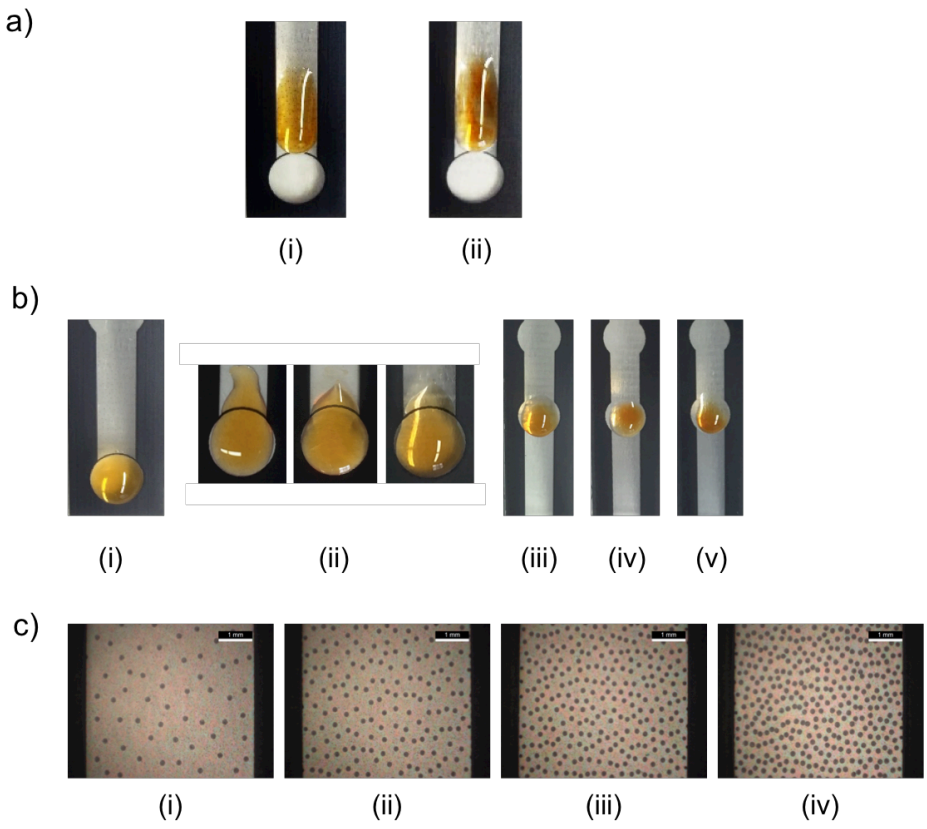

Figure S3: Optimization of wax dot printing between the 3rd area and the sensing area: a) Photographs visually showing the distribution of reagents in the case of (i) wax dots printed at 1% printing opacity setting (uniform mixing of the reagents in the sample droplet); (ii) absence of wax dots (incomplete mixing of the reagents in the sample droplet). b) Photographs after sample application to devices with various printing opacity settings: (i) 1% opacity (formation of hemispherical sample droplet in the sensing area); (ii) no opacity (*i.e.* droplet distortion, incomplete entrance of sample into the sensing area); (iii) 3% opacity; (iv) 5% opacity; (v) 7% opacity (retention of sample in the 3rd area due to excessive flow resistance at ≥3% opacity). c) Microscope pictures of wax dot-modified channel sections with various printing opacity settings: (i) 1% opacity; (ii) 3% opacity; (iii) 5% opacity; (iv) 7% opacity (scale bars: 1 mm). Contrast and brightness have been modified for the sake of visibility in part a and parts b (iii), (iv) and (v).

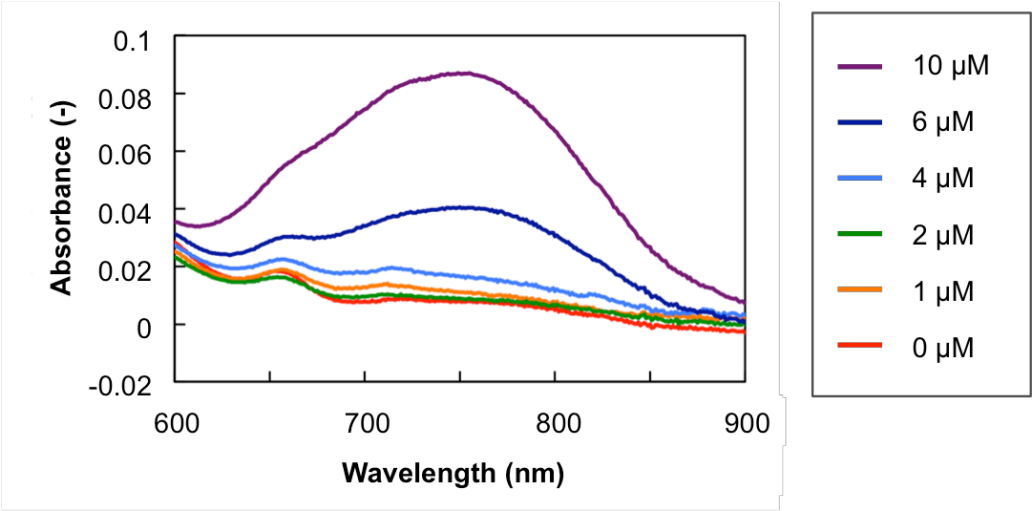

Figure S4: Absorbance spectra obtained from spot tests on an OHP film. A small peak/shoulder not attributed to the metal-nitroso-PSAP complex is observed around 650 nm.

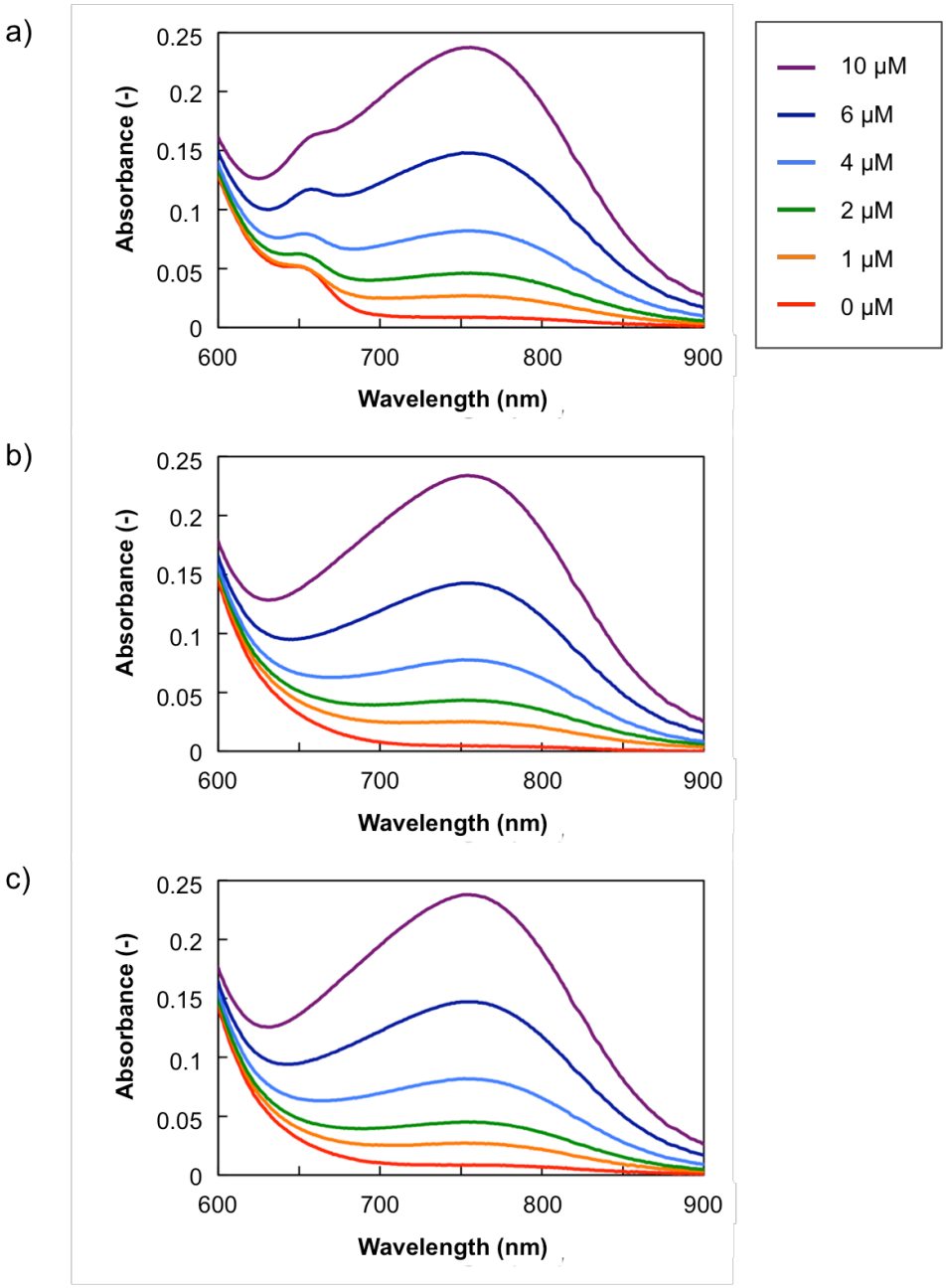

Figure S5: Absorbance spectra of Fe^{2+}-nitroso-PSAP complexes obtained under different reagent pre-mixing conditions in a 96-well microplate: a) ascorbic acid and nitroso-PSAP pre-mixed (condition (i) on page S4); b) phosphate buffer and nitroso-PSAP pre-mixed (condition (ii) on page S4); c) ascorbic acid and phosphate buffer pre-mixed (condition (iii) on page S4).

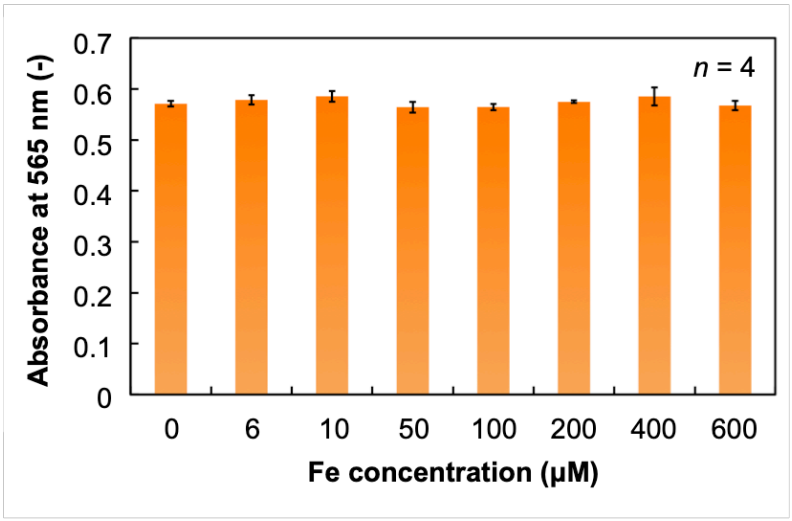

Figure S6: Iron concentration-dependent absorbance of acid red 52 at 565 nm on reagent-free µTFADs; each data point has been obtained by measurements with 4 individual single-use devices; error bars indicate the standard deviations.

96-Well microplate test to estimate the limit of detection (LOD) for iron ions based on 3σ method

To estimate the LOD of the iron ion assay on a microplate, reagents ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, ascorbic acid, phosphate buffer and nitroso-PSAP) were added to the microplate wells in the order shown below:

(i) $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$

(ii) ascorbic acid

(iii) pH 6.8 phosphate buffer

(iv) nitroso-PSAP

This order corresponds to the order of reagent dissolution in the case of the flow-based μTFAD assay. 50 μL of the final resulting mixture composed of ascorbic acid (4 mM), pH 6.8 phosphate buffer (10 mM), nitroso-PSAP (1 mM), and Fe^{2+} (0, 1, 2, 4, 6, 10 μM) was transferred to a separate microplate well and absorbance at 756 nm was immediately acquired by the microplate reader. The result is shown in Fig. S7.

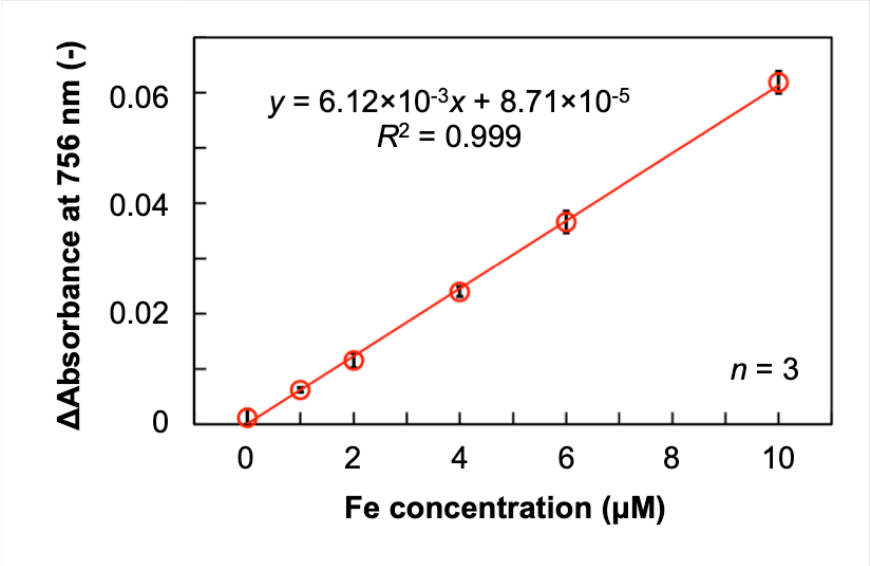

Figure S7: Calibration curve for 96-well microplate-based Fe^{2+} assay; error bars indicate standard deviations.

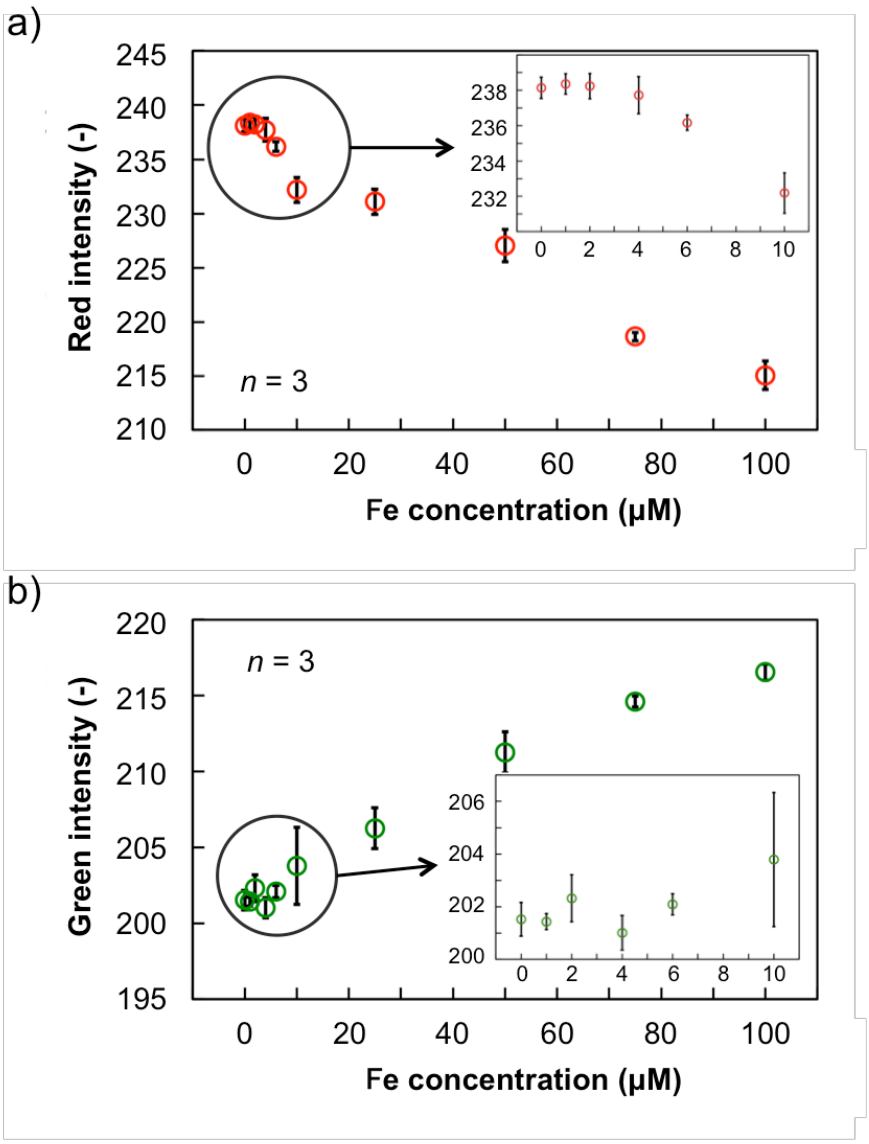

Figure S8: Sample iron concentration-dependent response on a paper substrate for a) red and b) green colour coordinates; each data point has been obtained by measurements with 3 individual single-use devices; error bars indicate the standard deviations.

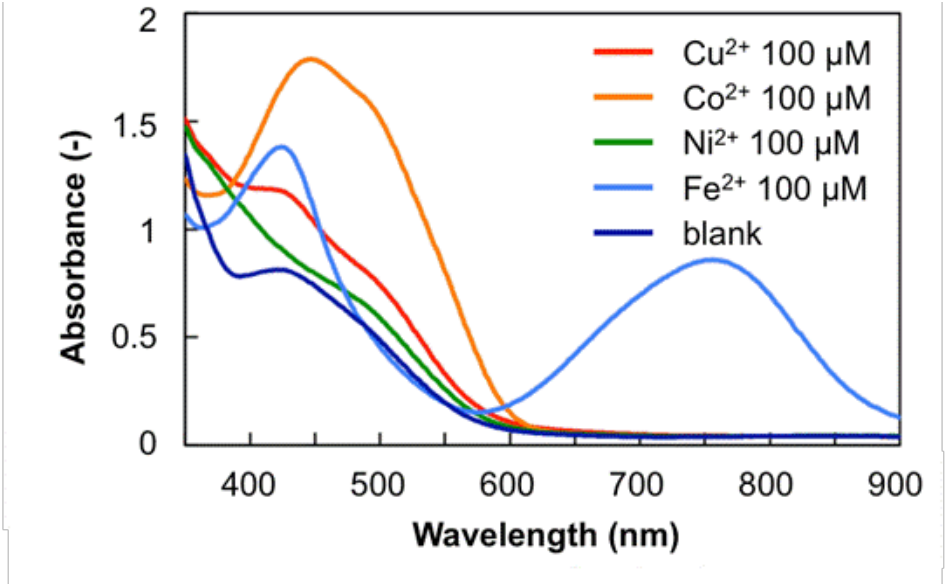

Figure S9: Original absorbance spectra of nitroso-PSAP in the presence of 100 µM of Cu^{2+} (red), Co^{2+} (yellow), Ni^{2+} (green), Fe^{2+} (aqua) and nitroso-PSAP alone (dark blue) acquired on the µTFADs.

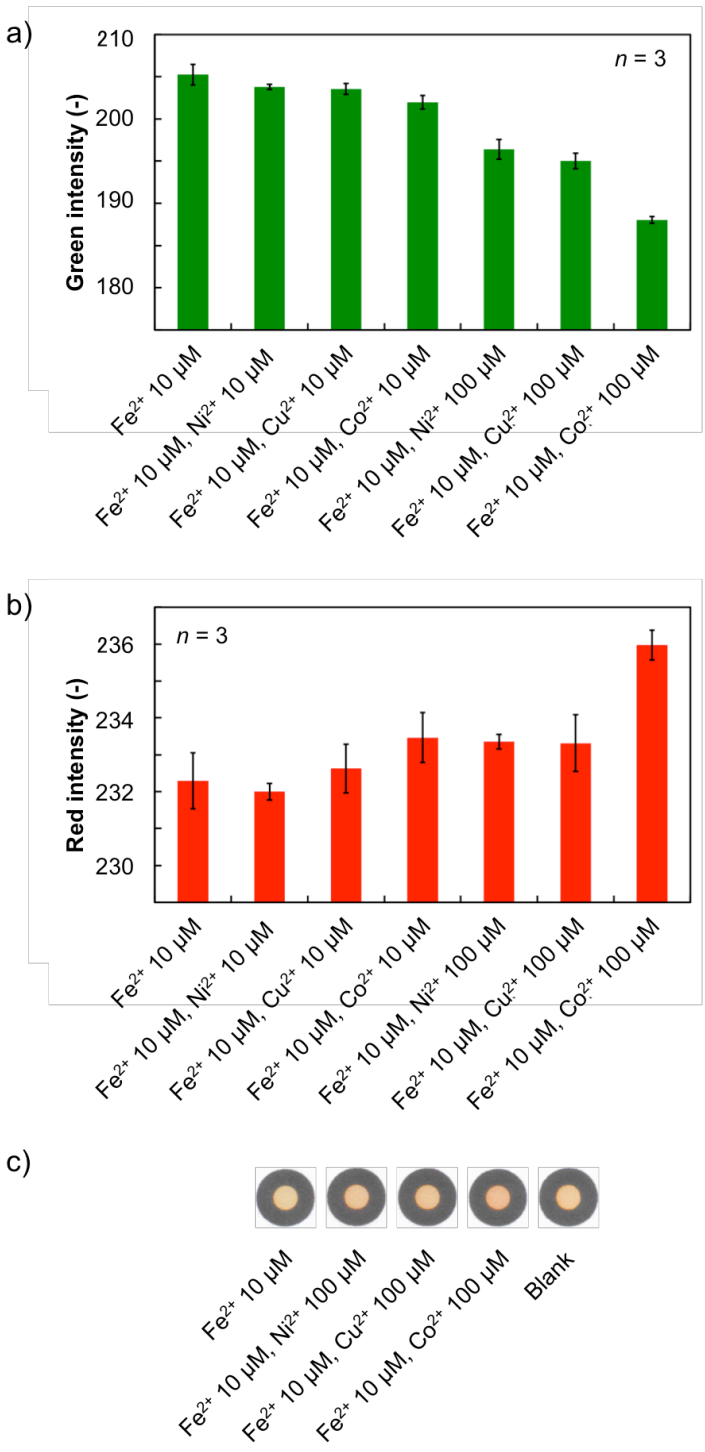

Figure S10: Interference of Cu^{2+}, Co^{2+} and Ni^{2+} ions with the Fe^{2+} assay based on the a) green and b) red colour intensity analysis of paper spots; each data point has been obtained by measurements

with 4 individual single-use devices; error bars indicate the standard deviations; c) Scanned images of nitroso-PSAP in the presence of 10 μM of Fe^{2+} and 100 μM of the interfering ions.