Supplementary Information

Ninety-six well plate as headspaces with moist starch indicator paper as cover for determination of ascorbic acid by iodate oxidation and formation of volatile iodine

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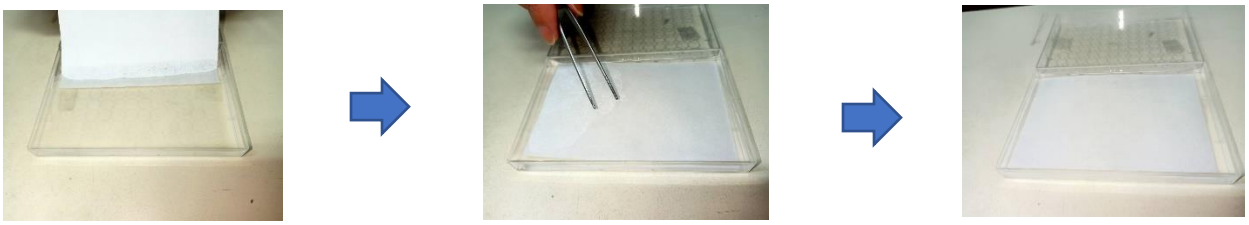
Supplementary Information A

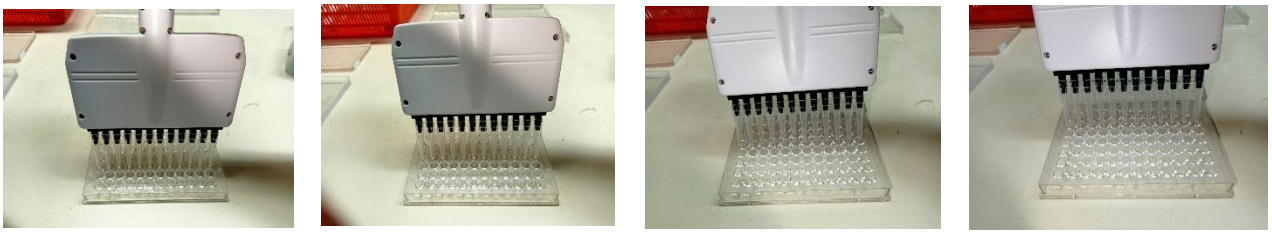
Stepwise procedure for preparation of starch indicator paper and the formation and measurement of starch-iodine complex using all 96 wells

The stepwise procedure for the formation and measurement of starch-iodine complex using all 96 wells is shown below.

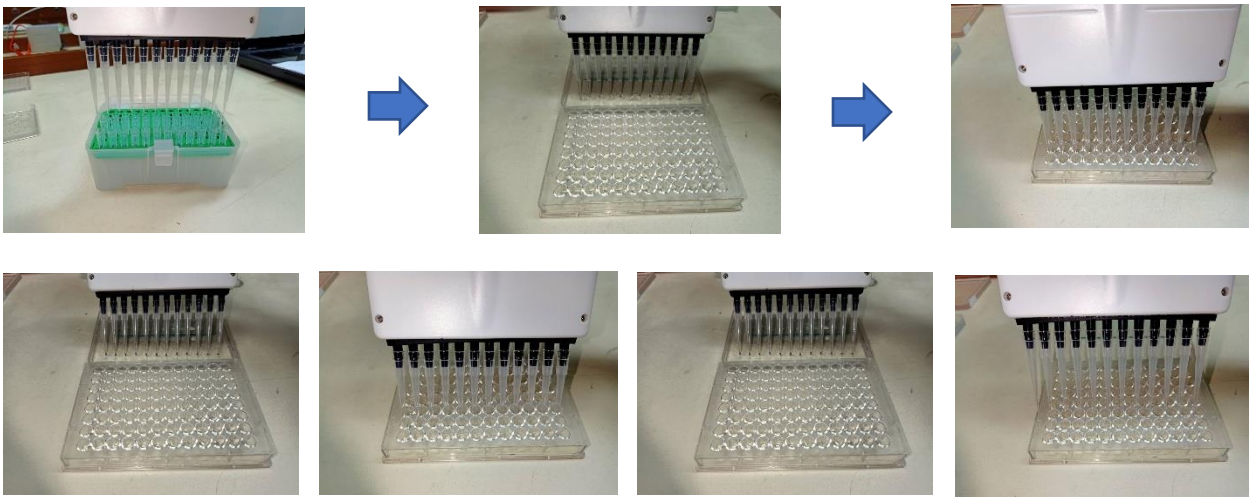
1. The sheet of chromatography paper (7.5 cm × 11.5 cm) is immersed in the 1.0 % w/v starch solution contained in the plastic cover of the 96-well plate. The paper is left to soak while the pipetting steps of the samples and reagents are carried out.



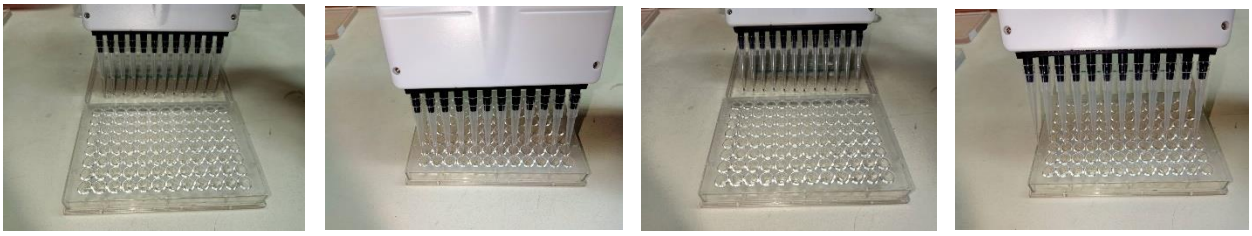
2. Aliquot (20 µL) of an ascorbic acid standard/sample solution is pipetted and dispensed simultaneously into 12 wells (one row) of the 96-well microplate using a 12-channel micropipette. This is repeated for the 2nd to the 8th standard/sample.



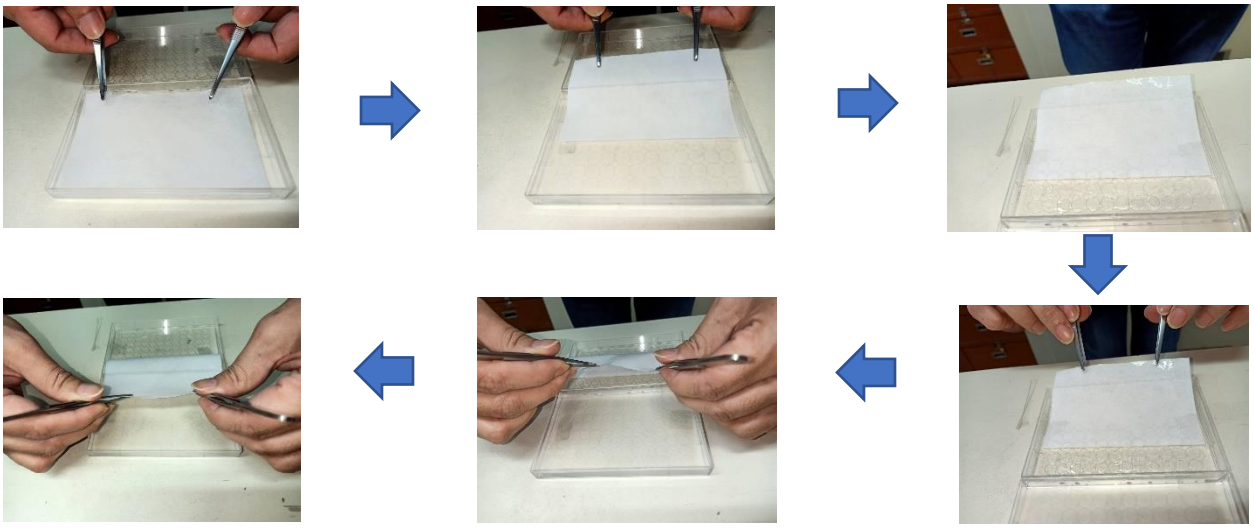
3. Then 20 µL aliquot of the 5 % aqueous acetic acid is added to all 96 wells using the 12-channel micropipette (8 pipetting/dispensing steps).



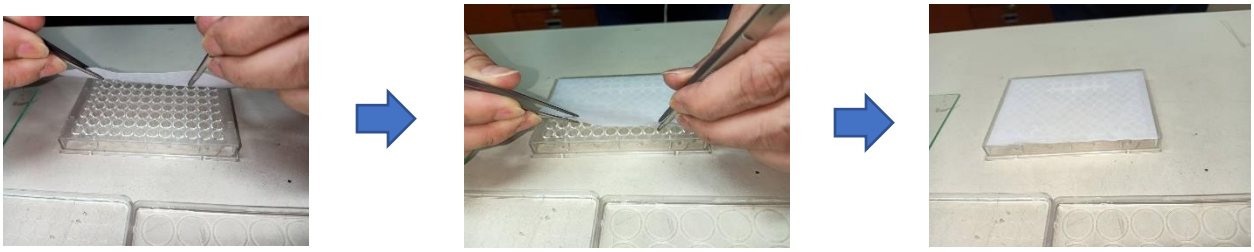
4. This is followed by 20 µL aliquots of 50 mM potassium iodate solution (time for step 4 *ca.*30 s)



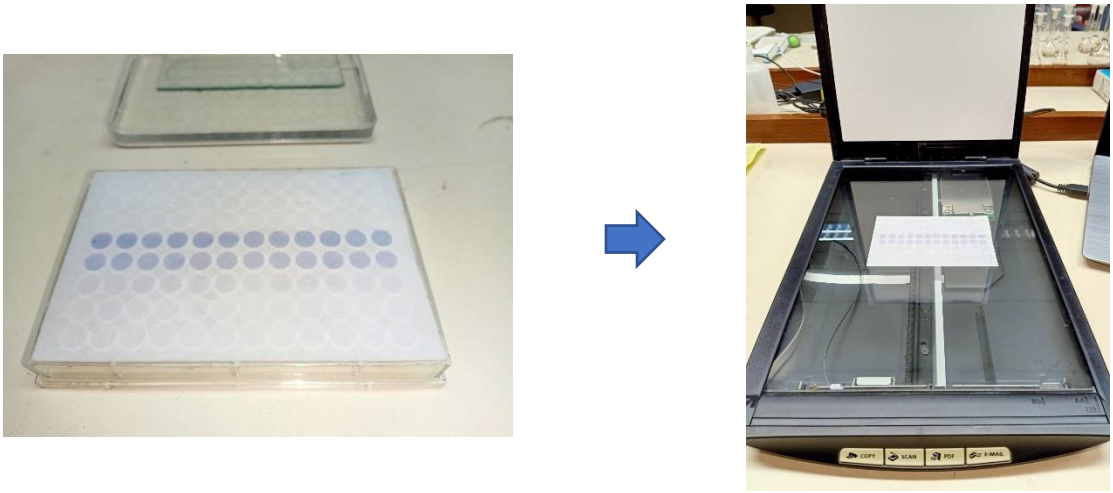
5. The starch paper is lifted out of the starch solution using two forceps gripping the paper strip lengthwise and drawing the surface of the paper against the top edge of the container. The step is repeated for the opposite side of the strip.



6. The strip of the moist indicator paper is then immediately placed over the 96-well plate. (time for steps 5-6 *ca.* 30 s)



7. Time period for iodine gas diffusion process is started. The covered 96-well plate is shaken for 10 s. After exactly 15 min, the indicator paper is removed and the side exposed to the iodine vapor scanned on a flatbed scanner.



Supplementary Information B

Calibration plots for absorbances of Red, Green and Blue values against concentrations of ascorbic acid

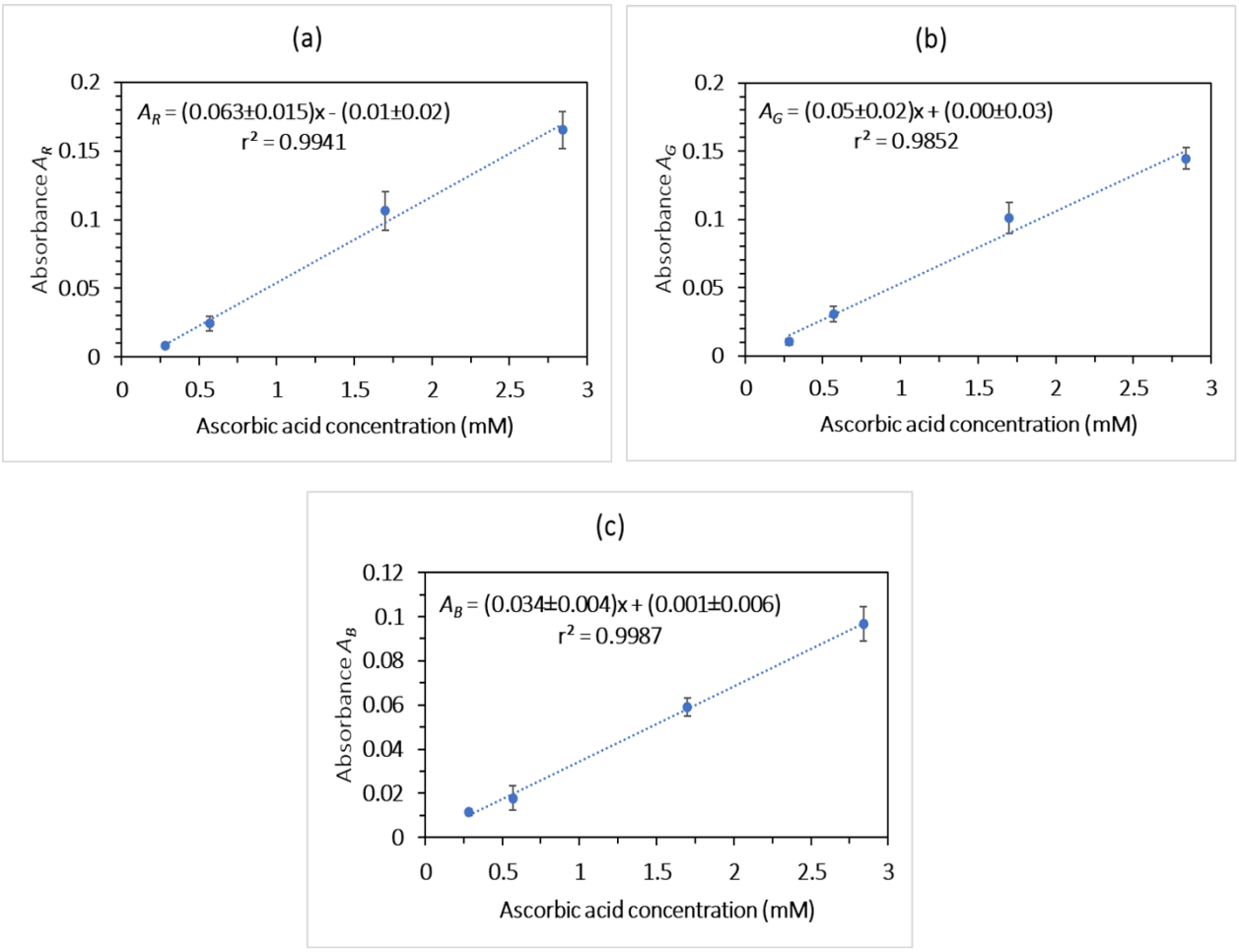


Fig. S1. Calibration plots of A_R (a), A_G (b) and A_B (c) with ascorbic acid concentrations. Error bars are the standard deviations from the mean *A* of the 12 wells. Experimental conditions: 0.284, 0.568, 1.70, and 2.84 mM ascorbic acid (20 μL); 5 % v/v acetic acid (20 μL; 50 mM KIO_3 (20 μL); 1.0 % w/v starch solution; 15 min measurement time. Procedure is shown in Fig. 1 and Supplementary Information A.

Table S1. List of the linear calibration equations, residuals sum of squares (RSS), coefficient of determination (r^2) and limit of detection (LOD) for the three color values.

Color	Linear calibration equation	RSS	r^2	LOD
Red	A_R = (0.063±0.015)x - (0.01±0.02)	9.59×10^{-5}	0.9941	0.35 mM
Green	A_G = (0.05±0.02)x + (0.00±0.03)	1.72×10^{-4}	0.9852	0.56 mM
Blue	A_B = (0.034±0.004)x + (0.001±0.006)	6.15×10^{-6}	0.9987	0.16 mM