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# Supplementary Information

# New and high-performance microfluidic analytical device based on Fusion 5 paper for the detection of chili pepper anthracnose pathogen *Colletotrichum truncatum*

Amir Osman Abdelrazig[1], Bao Thai Tran[1], Patsamon Rijiravanich[2*], Werasak Surareungchai[1, 3**]

[1]School of Bioresources and Technology, King Mongkut´s University of Technology Thonburi, Bang Khun Thian, Bangkok 10150, Thailand.

[2]Bioscience and System Biology Research Team, National Center for Genetic Engineering and Biotechnology, National Sciences and Technology Development Agency at King Mongkut´s University of Technology Thonburi, Bang Khun Thian, Bangkok 10150, Thailand.

[3]Nanoscience and Nanotechnology Graduate Program, Faculty of Science, King Mongkut´s University of Technology Thonburi, Pracha Uthit Rd, Bangkok 10140, Thailand.

Corresponding author Email: *patsamon.rij@biotec.or.th, **werasak.sur@kmutt.ac.th

ORCID: 0000-0002-7080-9513 (Rijiravanich, P.), 0000-0002-3639-7279 (Surareungchai, W.)

## Method

Figure S1. shows the location of forward and reverse primers used for PCR amplification compare to capture and reporter probe used for detection of *Colletotrichum truncatum* on ACT-Ct-PAD.

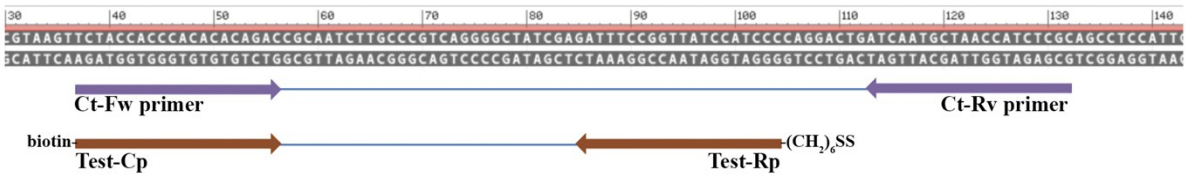


**Figure S1.** The location of the primers in the violet arrows for Ct-Fw and Ct-Rv primers of *C. truncatum* and dark orange arrows for test-capture probe and test -reporter probe.

**Results**

**The wax-patterned fusion 5 sensor**

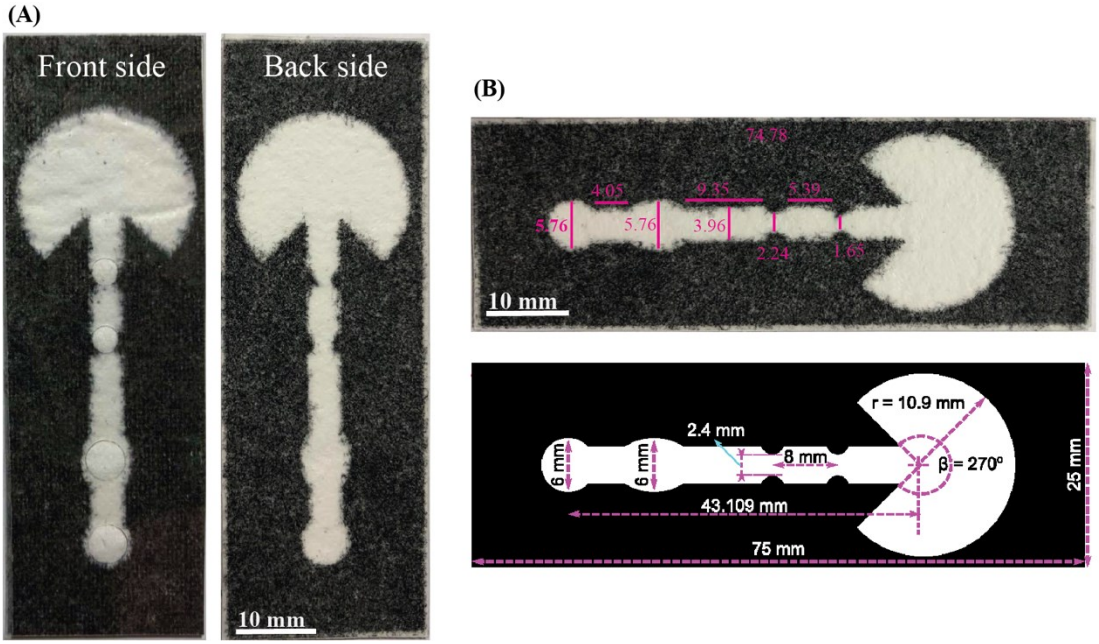


**Figure S1**. Image of wax patterned fusion 5 paper (A) front and back sides, and (B) with scaling and compared to the designed pattern (bottom image).

**Localization of MSP on test and control zone**

The particles deposited onto the test and control zone was confirmed by red color of AuNPs represent at the test and control spots. By the way, we did some experiments to confirm the localization of the particles-capture probe onto the test and control zones. We held the following experiment:

1 mL of 1 mg $mL^{-1}$ of the redox dye MTT (*3*-(*4*,*5*-Dimethylthiazol-*2*-yl)-*2*,*5*-Diphenyltetrazolium Bromide) with a yellow color was encapsulated into 10 mg of mesoporous silica particles (MSP) by physical adsorption overnight. The suspension was then centrifuged and the pellet washed 5 times with milli-Q water and dried overnight in silica box. To confirm that dye is well encapsulated and there is no leakage, MSP-MTT was dispersed in milli-Q water and incubated overnight at room temperature. The dispersion was centrifuged at 5,000 rpm for 5 min and supernatant was transferred to a clean tube. To 200 µL of the supernatant, 10 µL of 1 M NaOH was further added. (NaOH at high concentration has a dual function: it causes bursting of MSP particles and release of the dye, and also reduce MTT tetrazolium dye to insoluble purple formazan product). Therefore, if there is a leakage of the dye from MSP the supernatant will turn purple. As shown in the Fig. S2(A) there was no color change in the supernatant, indicating a good encapsulation of the dye without any leakage.

Further, MSP-MTT was coated with polyelectrolytes and avidin in the same way we shown in material and methods section. 1 µL of the coated particles was dispensed on the

test and control zones of the device and allowed to dry in silica box for 30 min at room temperature. 100 µl of PBS (sample buffer) was applied to the sample area and flow until the absorbent area. The device was allowed to dry in silica box for 1 h, then, 2 µL of 1 M NaOH was added to the test and control spots. Furthermore, 10 µl NaOH was added immediately upstream of the test and control spots. According to the Fig. S2(B), the purple color was observed only in the test and control spots, indicating the localization of the particles in these spots.

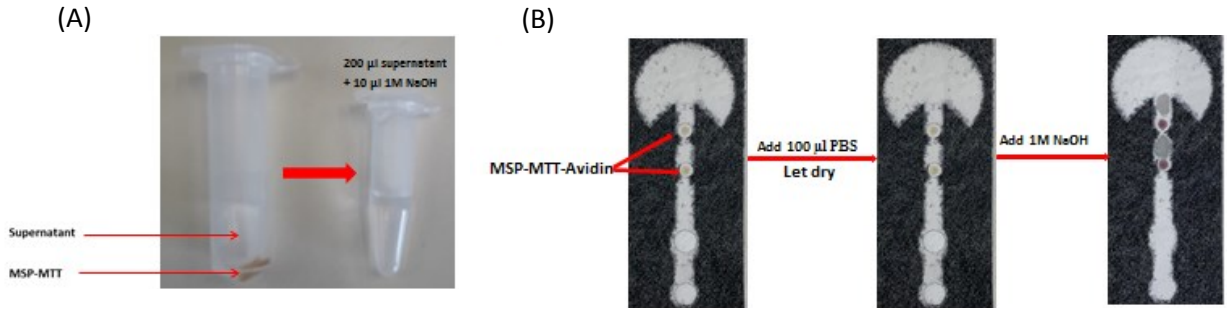


**Figure S2**. (A) The image of MSP-MTT pellet derived after centrifugation at 5,000 rpm for 5 min and testing the leakage of MTT from MSP by adding NaOH in the supernatant. (B) shows the image of Fusion5-PAD dropped MSP-MTT at test and control zone with the step by step testing with flowing of PBS and add 1 M NaOH to develop the color.

**Optimization conditions for ACT-Ct-PAD**

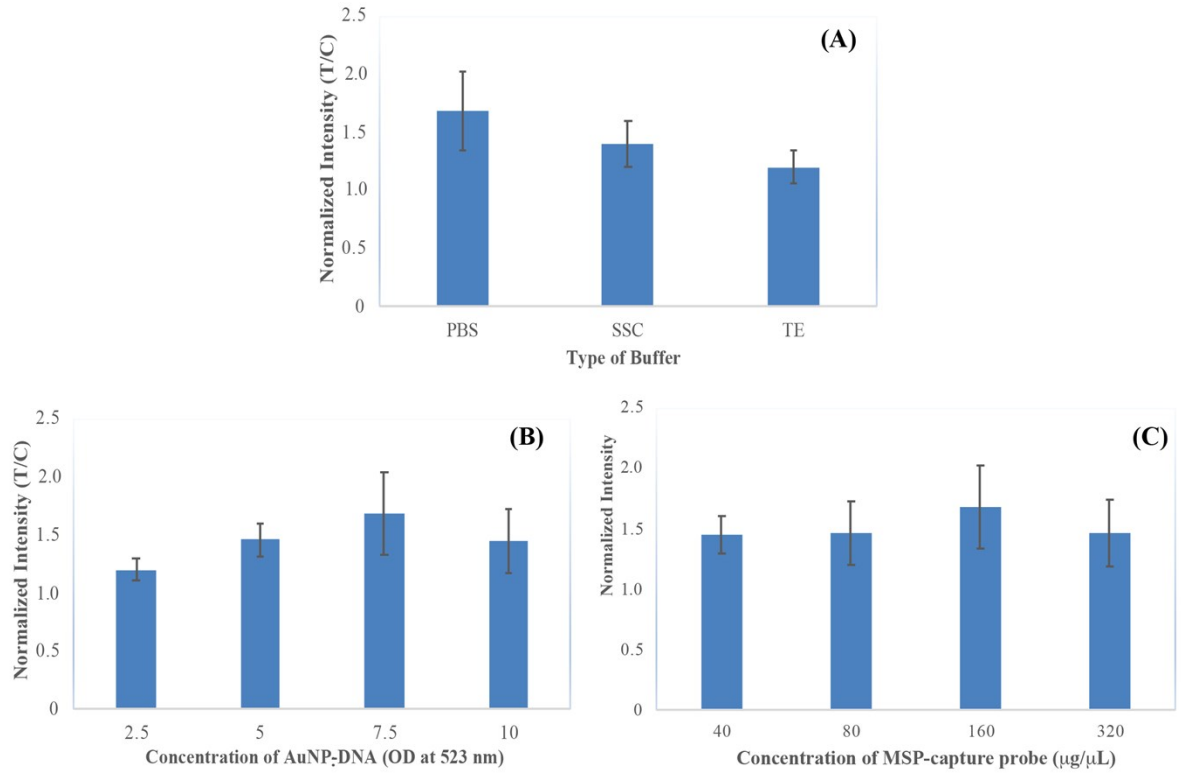


**Figure S3**. Signal responses from the optimized conditions of (A) type of buffer (PBS = 0.01 M PBS, pH 7.4, SSC = 1x SSC buffer, pH 8.0 and TE = 10 mM *Tris*-HCl containing 1 mM EDTA, pH 8), (B) concentration of AuNPs-DNA label and (C) concentration of MSP-capture probes. The standard deviation derived from *n* = 19.

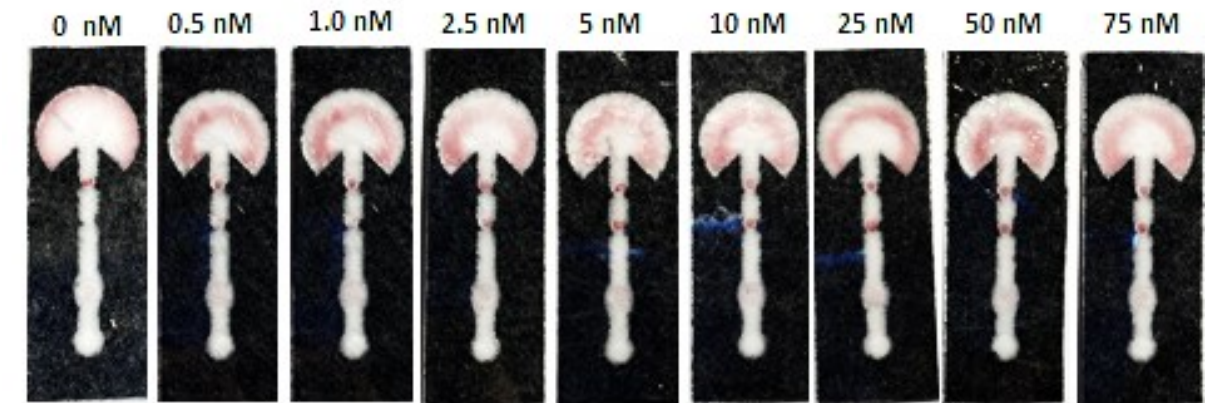


**Figure S4.** Image of the devices tested with different concentrations of 69 mer oligonucleotide target.