

1 **Supplementary information**

2 **Improving design features and air bubble manipulation techniques for a single-step sandwich**
3 **electrochemical ELISA incorporating commercial electrodes into capillary-flow driven**
4 **immunoassay devices**

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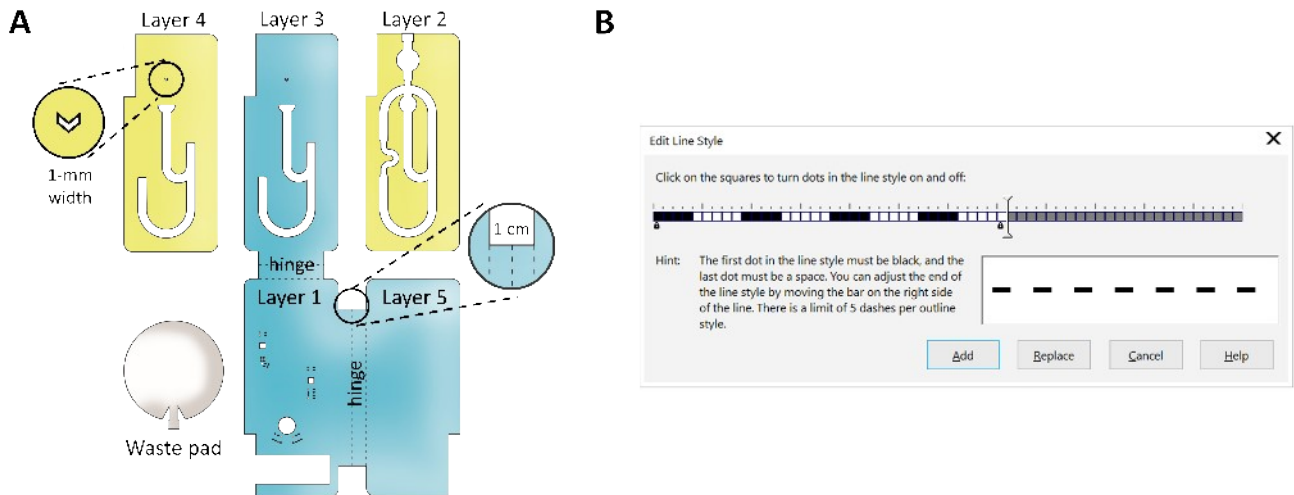
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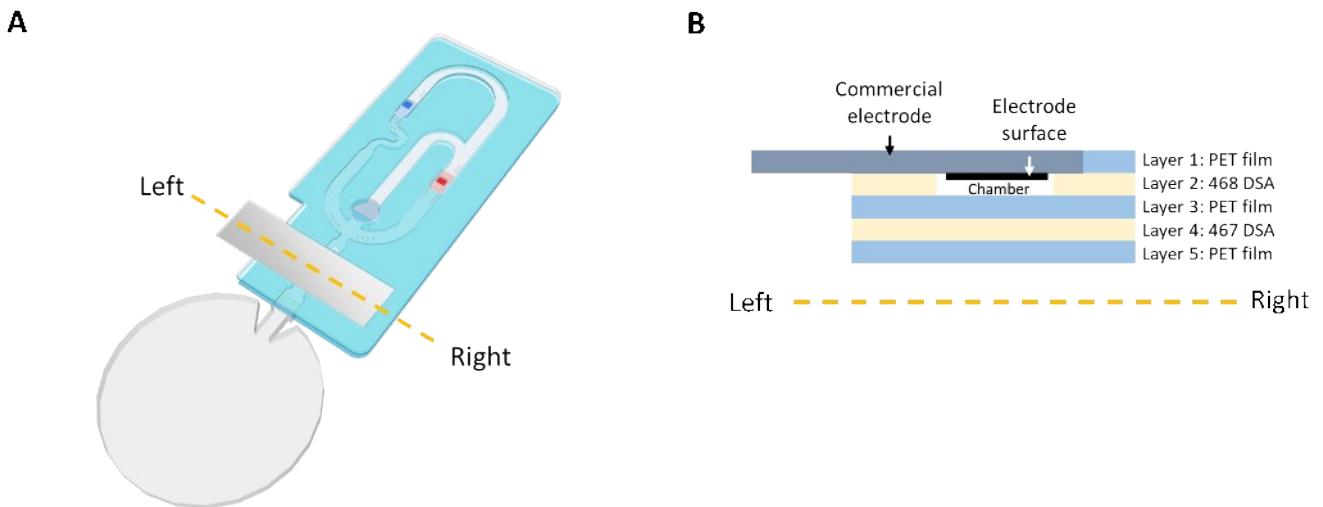
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1 SI Results

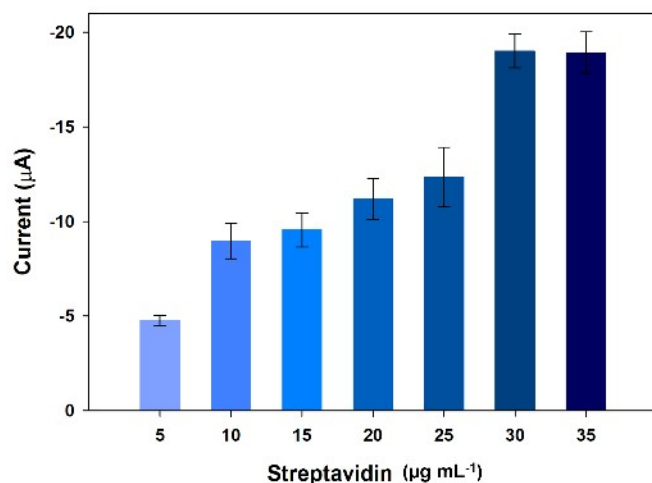


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3 **Figure S1.** The iceCaDI device design, A) the layout and details, B) the setting of the dashed line for the
4 foldable hinge.

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7 **Figure S2.** A) The iceCaDI device after the final assembly and B) the cross-section area at the electrode
8 chamber.

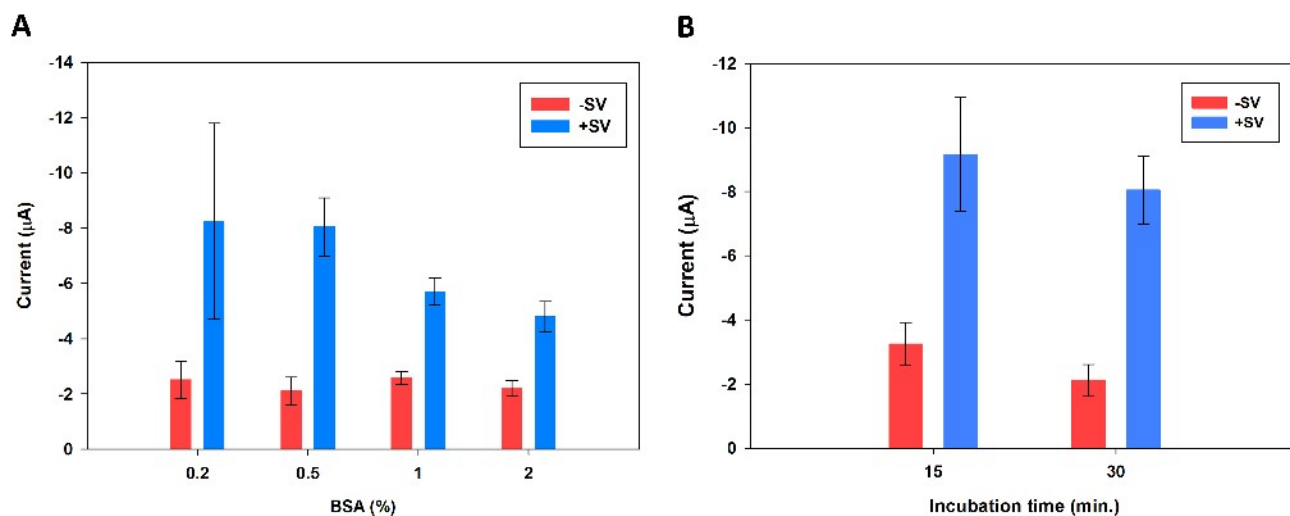


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2 **Figure S3.** The optimization of streptavidin concentration using 25 µg mL⁻¹ of HRP (biotin) and 0.5%
 3 BSA for blocking.

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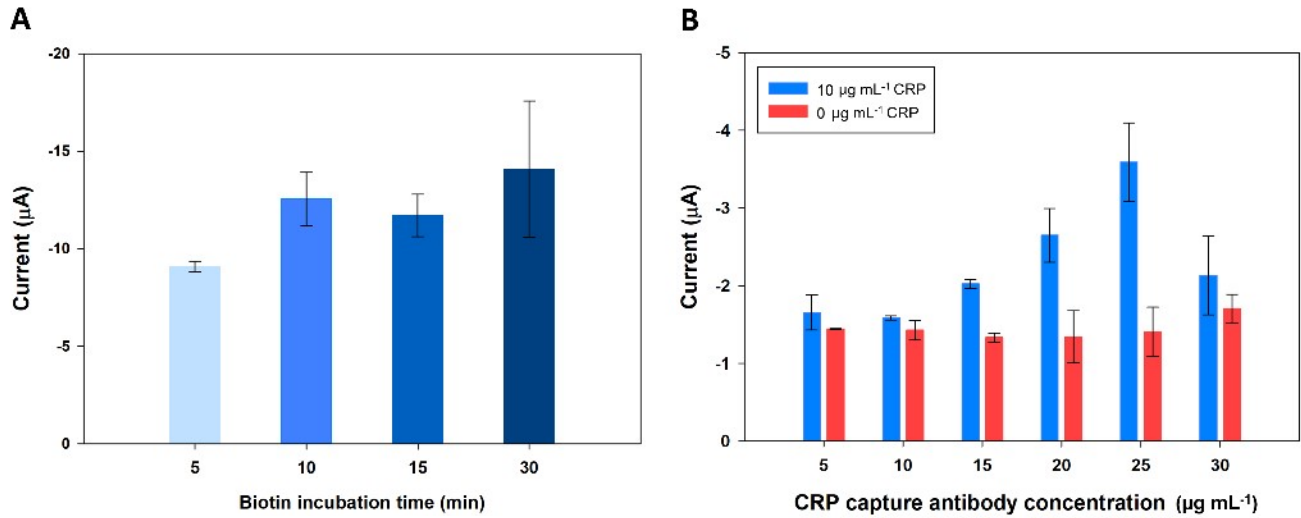


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7 **Figure S4.** The optimization of blocking using BSA with or without 25 µg mL⁻¹ of streptavidin (SV), A)
 8 The optimization of BSA concentration at 30 min of blocking time, B) The optimization of blocking time.

9 All experiments were obtained using 25 µg mL⁻¹ of HRP (biotin).

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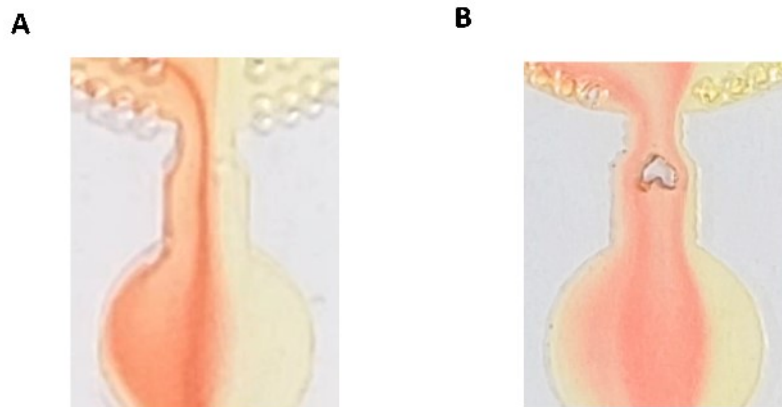


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2 **Figure S5.** The optimization of CRP capture antibody (biotin) immobilization. A) The optimization of
 3 biotin and streptavidin binding time obtained using 25 µg mL⁻¹ of HRP (biotin), B) The optimization of
 4 CRP capture antibody (biotin) concentration with or without 10 µg/mL of CRP. All experiments were
 5 performed with 25 µg mL⁻¹ of streptavidin for 1 hr and 0.5% BSA blocking for 30 min.

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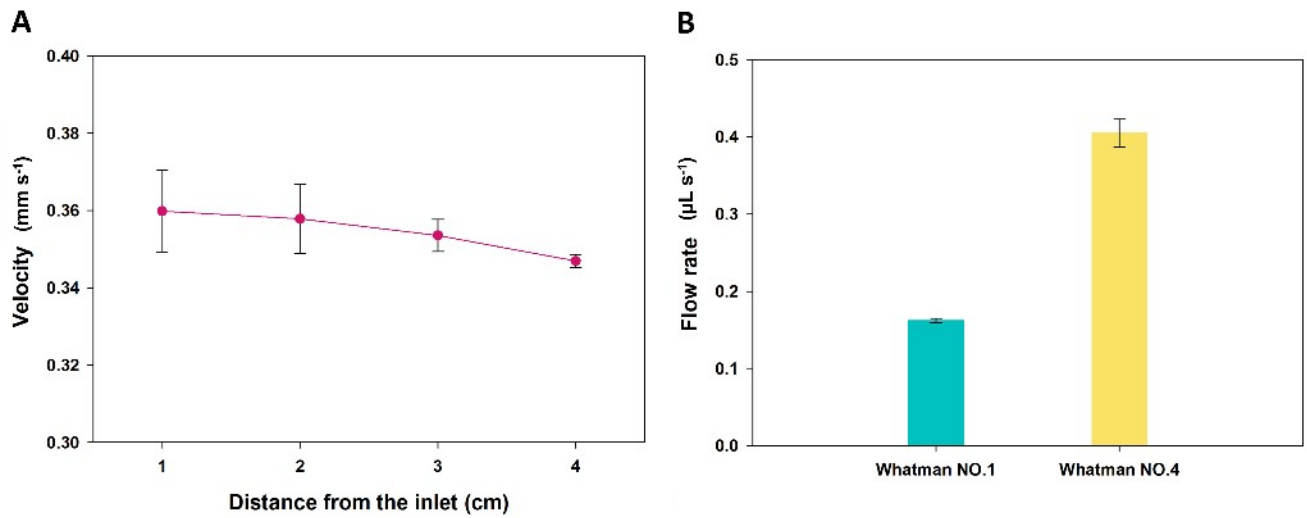
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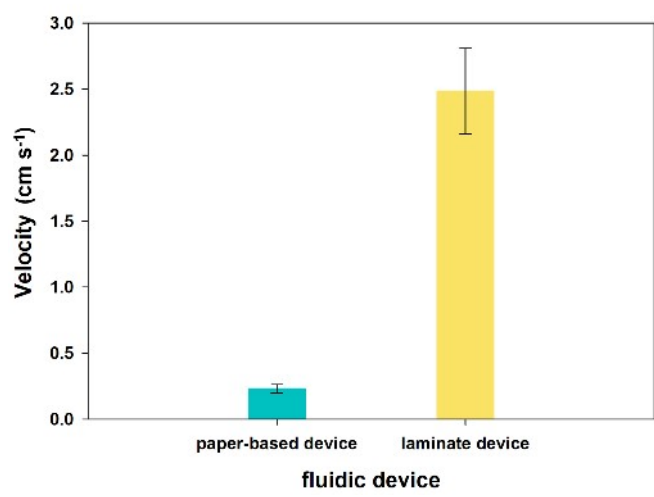
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9 **Figure S6.** The flow in electrode chamber comparison, A) without the arrow-shaped obstacle, B) with the
 10 arrow-shaped obstacle.

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 2 **Figure S7.** The flow study of the waste pad used, A) the velocity driven by the waste pad (Whatman
 3 No.4.) over distance from the inlet, B) the flow rate comparison of the waste pad using Whatman No.1 vs
 4 Whatman No.4 filter paper.
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 7 **Figure S8.** Velocity comparison of paper-based (nitrocellulose strip; CN 95) and laminated devices with
 8 3-mm wide and 4-cm long channels. The velocity was measured at a 4-cm distance from the inlet.
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