

# Advanced Detection of Cervical Cancer Biomarkers Using Engineered Filamentous Phage Nanofibers

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**Table S1.** P/N value of Phage sandwich-ELISA checkerboard.

| Capture antibody: fd-LC-WT | Detection antibody: mix1 | Mix 2       | Mix 3 | Mix 4 | Mix 5 | Mix 6 | Mix 7 | Mix 8 | Mix 9 | PBS  |
|----------------------------|--------------------------|-------------|-------|-------|-------|-------|-------|-------|-------|------|
| 25ug/ml                    | 2.87                     | 2.71        | 2.82  | 2.86  | 2.81  | 2.96  | 2.75  | 2.48  | 2.29  | 0.92 |
| 50ug/ml                    | 3.69                     | <b>3.91</b> | 3.29  | 2.69  | 2.98  | 3.323 | 2.87  | 2.39  | 2.28  | 0.99 |
| 75ug/ml                    | 3.05                     | 3.34        | 3.66  | 3.05  | 3.28  | 3.29  | 2.81  | 2.75  | 2.43  | 0.99 |
| 100ug/ml                   | 3.48                     | 3.34        | 3.29  | 3.68  | 3.40  | 3.74  | 2.47  | 2.88  | 1.97  | 0.92 |

**Table S2.** The mixture of different dilutions of anti-VEGF and anti-PD-L1 antibodies

| Dilution   | anti-VEGF | 1/2 500 | 1/ 5 000 | 1/ 10000 |
|------------|-----------|---------|----------|----------|
| anti-PD-L1 | 1/2 500   | Mix 1   | Mix 2    | Mix 3    |
|            | 1/ 5 000  | Mix 4   | Mix 5    | Mix 6    |
|            | 1/ 10000  | Mix 7   | Mix 8    | Mix 9    |

**Table S3.** Inter-batch reproducibility test of phage sandwich-ELISA.

| Sample | OD450 |      | cv%  |       |
|--------|-------|------|------|-------|
| P1     | 0.78  | 0.90 | 0.88 | 6.15  |
| p2     | 0.67  | 0.79 | 0.73 | 7.11  |
| p3     | 0.7   | 0.83 | 0.77 | 6.84  |
| p4     | 1.17  | 1.48 | 1.27 | 9.92  |
| p5     | 0.92  | 1.14 | 0.89 | 11.1  |
| p6     | 0.74  | 0.88 | 0.81 | 7.38  |
| N 1    | 0.36  | 0.44 | 0.43 | 9.01  |
| N 2    | 0.28  | 0.3  | 0.33 | 10.04 |
| N 3    | 0.40  | 0.54 | 0.47 | 11.58 |

\*The repeatability of Phage ELISA kits was expressed as a coefficient of variation (CV) of results of sera tested 3 times in different days by the period of 1 month, by the same samples (serum from 6 cervical cancer patients and 3 healthy controls), in the same conditions, and by using the same equipment.

**Table S4.** Intra-batch reproducibility test of phage sandwich-ELISA.

| Sample | OD450 |      |      | cv%  |
|--------|-------|------|------|------|
| P1     | 0.78  | 0.80 | 0.76 | 2.48 |
| p2     | 0.67  | 0.73 | 0.74 | 4.41 |
| p3     | 0.69  | 0.69 | 0.72 | 2.15 |
| p4     | 1.17  | 0.98 | 0.99 | 8.36 |
| p5     | 0.92  | 0.87 | 0.88 | 2.45 |
| p6     | 0.74  | 0.69 | 0.75 | 3.56 |
| N 1    | 0.36  | 0.34 | 0.36 | 3.56 |
| N 2    | 0.28  | 0.3  | 0.29 | 3.07 |
| N 3    | 0.40  | 0.37 | 0.39 | 4.95 |

\* The repeatability of Phage ELISA kits was expressed as a coefficient of variation (CV) of results of sera tested 3 times in same day, by the same samples, in the same conditions, and by using the same equipment.

Figure S1. Detection of serum sPD-L1 by Phage sandwich-ELISA Method. 16 healthy human sera samples (HP), 25 precancerous cervical lesion samples (PCL), and 71 cervical cancer samples (CC) were studied in this article. (A) The concentration of sPD-L1 in group of CC is significantly higher than the group of PCL and HP. (\*  $P < 0.05$ , \*\*  $P < 0.01$ ).; (B) The area under the ROC curve (AUC) for serum sPD-L1 was 0.778 when using the phage-ELISA method in the cohort of 71 CC patients and 16 healthy controls. (\*\*  $P < 0.01$ ).

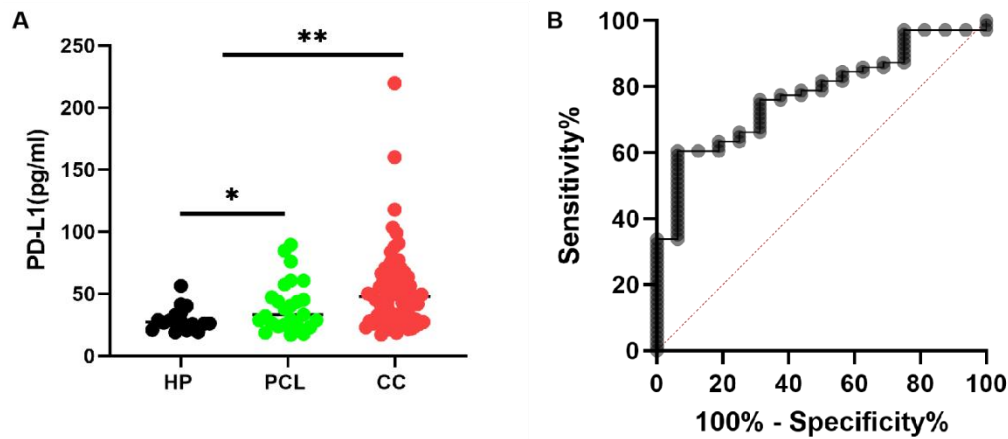


Figure S2. Detection of serum VEGF by Phage sandwich-ELISA Method. 16 healthy human sera samples (HP), 25 precancerous cervical lesion samples (PCL), and 71 cervical cancer samples (CC) were studied in this article. (A) The concentration of VEGF in group of CC is significantly higher than the group of HP. (\*  $P < 0.05$ , \*\*  $P < 0.01$ ).; (B) The area under the ROC curve (AUC) for serum VEGF was 0.756 when using the phage-ELISA method in the cohort of 71 CC patients and 16 healthy controls. (\*\*  $P < 0.01$ ).

