# Supplemental

## Carbohydrate binding module-fused antibodies improve the performance of cellulose-based lateral flow immunoassays

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**Keywords**

Lateral flow assay, point-of-care diagnostics, Covid-19 antibody test, pregnancy detection, carbohydrate-binding module, cellulose, sustainability, SARS-CoV-2

# Supplementary Figures

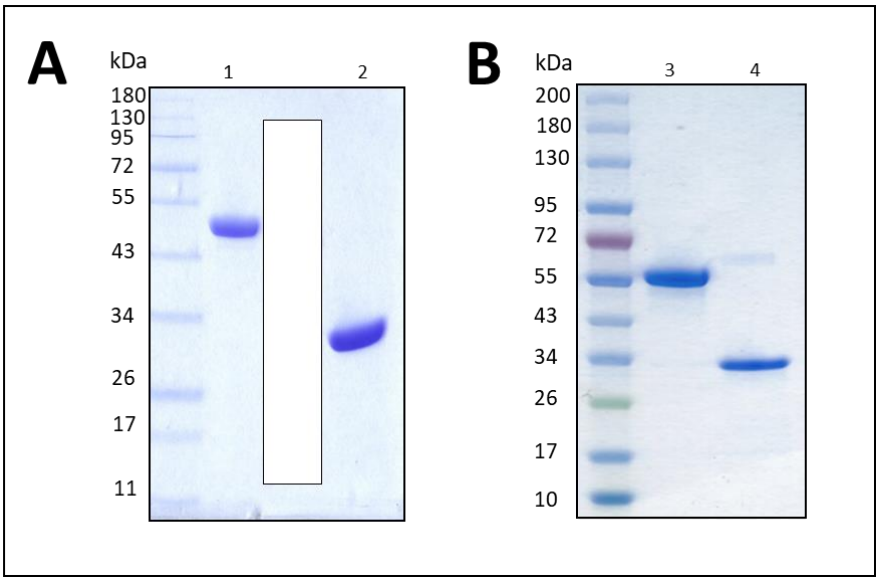


**Figure S1. SDS-PAGE and Coomassie staining analysis with the purified protein samples used for the generation of LFTs for the detection of (A) hCG and (B) SARS-CoV-2 specific antibodies.** (1) CBM-anti-hCG-scFv; (2) anti-hCG scFv; (3) CBM-anti-Fc scFv; (4) anti-Fc scFv. (**A**) The protein ladder (Blue Prestained Protein Standard Broad Range; New England Biolabs) and (**B**) Color Prestained Protein Standard Broad Range; New England Biolabs) was used.

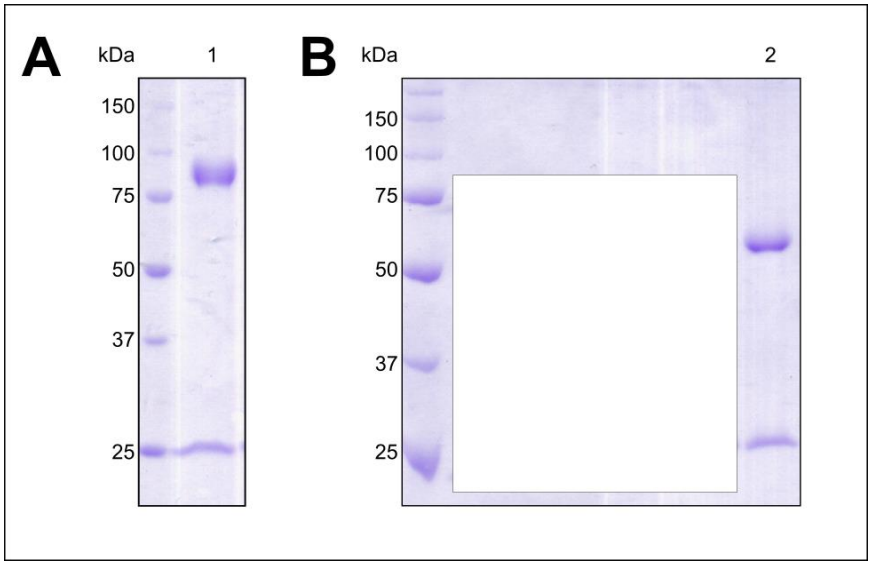


**Figure S2. SDS-PAGE and Coomassie staining analysis of protein A affinity purified full-length IgG and IgG-CBM fusion protein samples used for the generation of LFTs for the detection of human SARS-CoV-2 specific antibodies.** (**A**) representative mIgG2a-CBM-HIS anti-human IgG; (**B**) representative mIgG2a anti-human IgG. The protein ladder Precision Plus Protein Unstained Standards (Bio-Rad) was used.

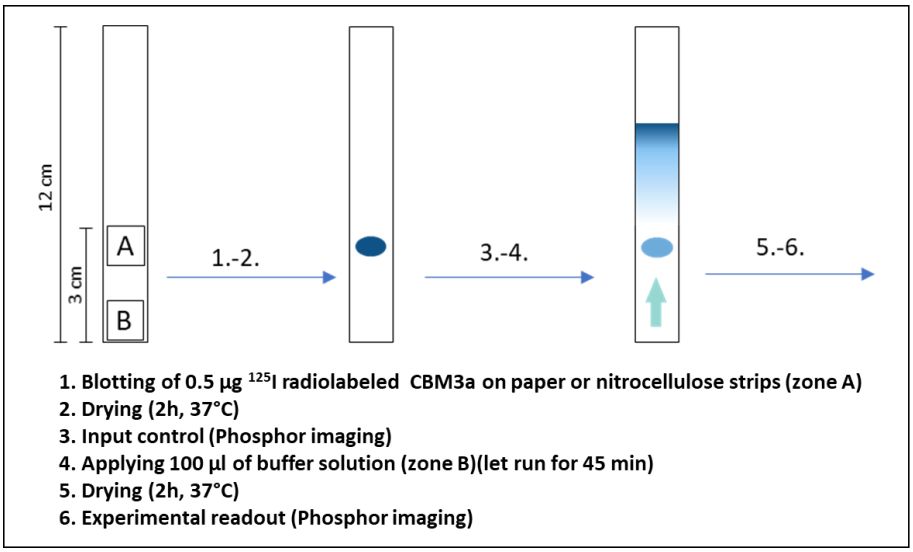


**Figure S3. Experimental setup and procedure, for the spatially resolved analysis of CBM binding on cellulose and nitrocellulose.** Detailed description can be found in the materials and methods section.

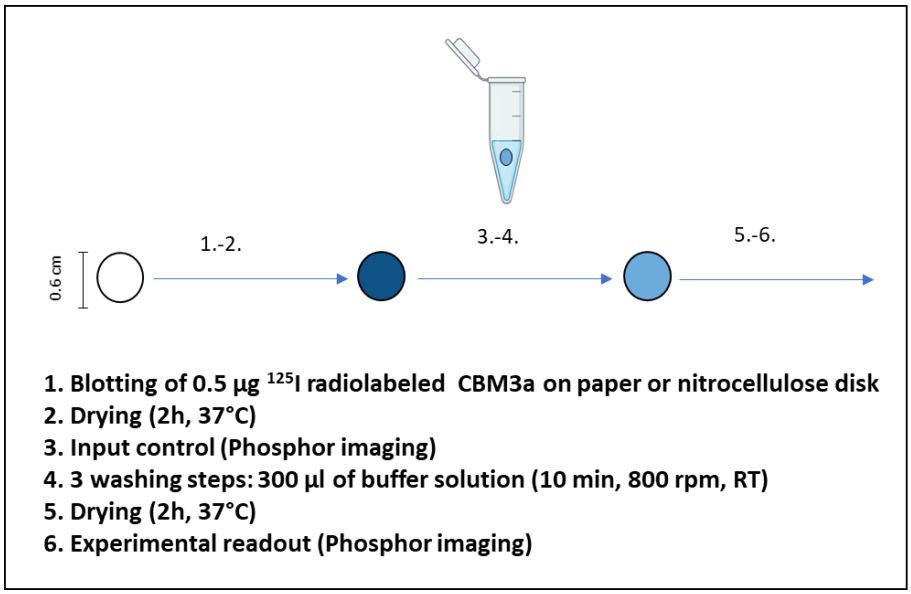


**Figure S4. Experimental setup and procedure, for the quantitative analysis of CBM binding on cellulose and nitrocellulose.** Detailed description can be found in the materials and methods section.

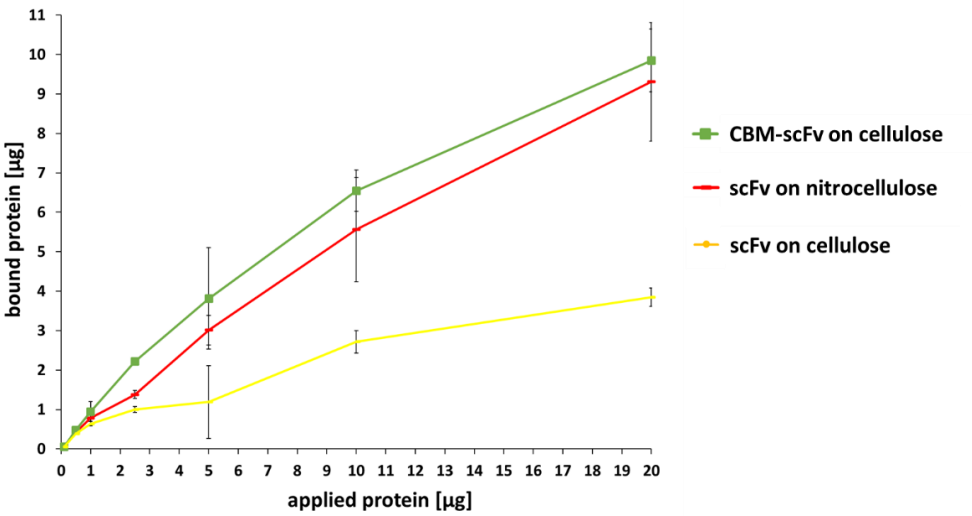


**Figure S5. Quantitative analysis of the protein binding capacity of CBM-scFv and the corresponding solitary scFv on cellulose in comparison to the binding capacity of the solitary scFv on nitrocellulose.** Experiments were performed according to Figure S6. Standard derivation was calculated using data derived from experimental triplicates.

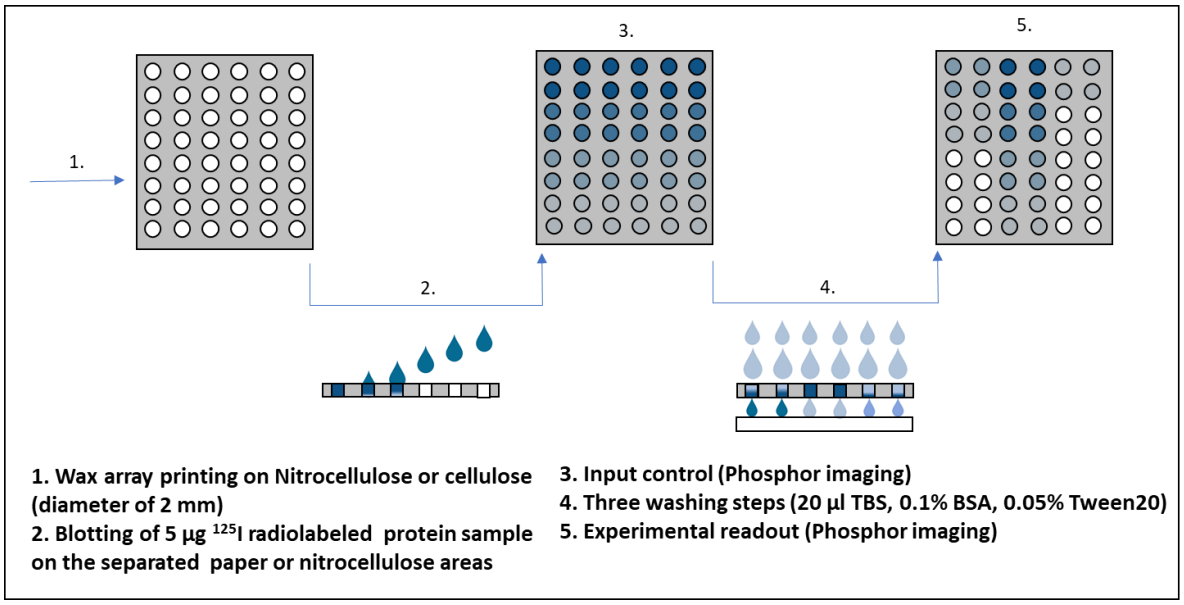


**Figure S6. Experimental setup and procedure, for the quantitative analysis of CBM, CBM fused molecules and non-fused molecules binding on cellulose and nitrocellulose.** Detailed description can be found in the materials and methods section.

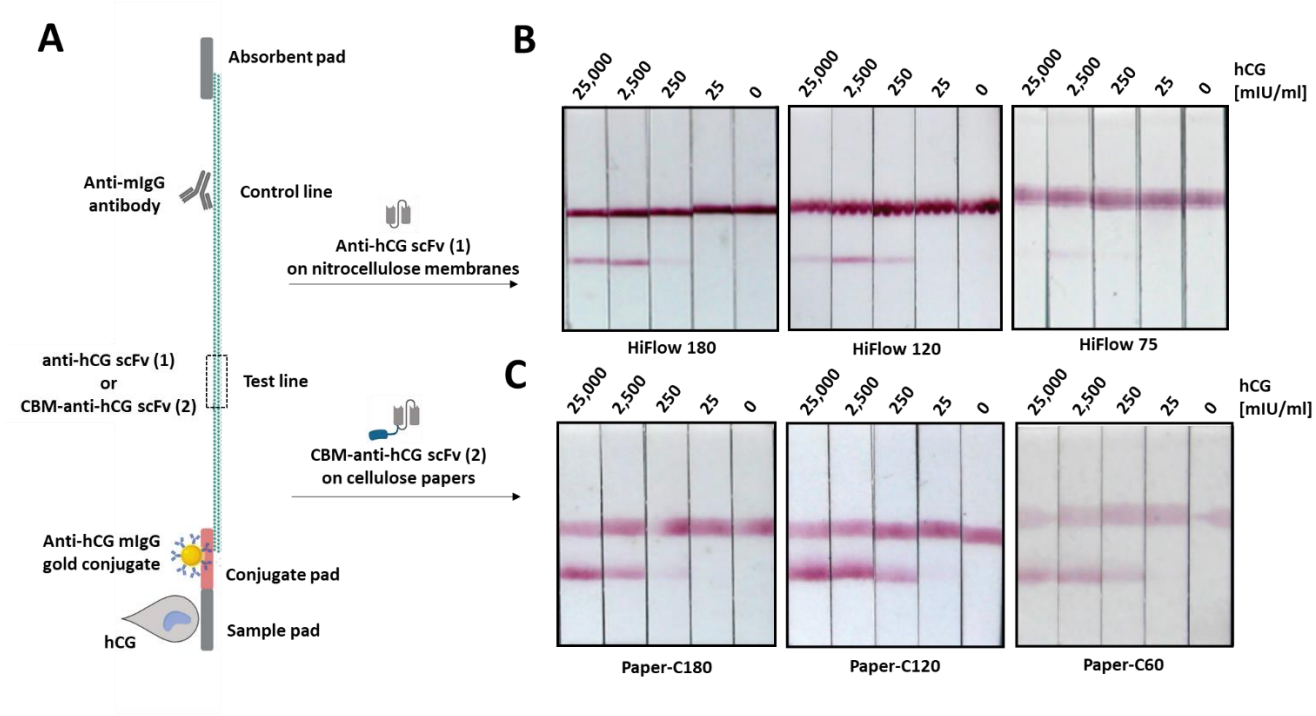


**Figure S7. LFTs for the detection of hCG using different cellulose-based papers (C180, C120, C60) and NC membranes (HiFlow180, HiFlow120, HiFlow75).** (**A**) Experimental setup for the LFT for pregnancy detection using (**B**) the solitary anti-hCG scFv on NC membranes or (**C**) CBM-anti-hCG scFv on cellulose paper. 150 µl of sample was applied to the sample pad, containing 150 µl synthetic urine with varying concentrations of hCG. Sample compositions are listed in Table S1. LFA experiments were performed in duplicates, all shown in Figure S9.

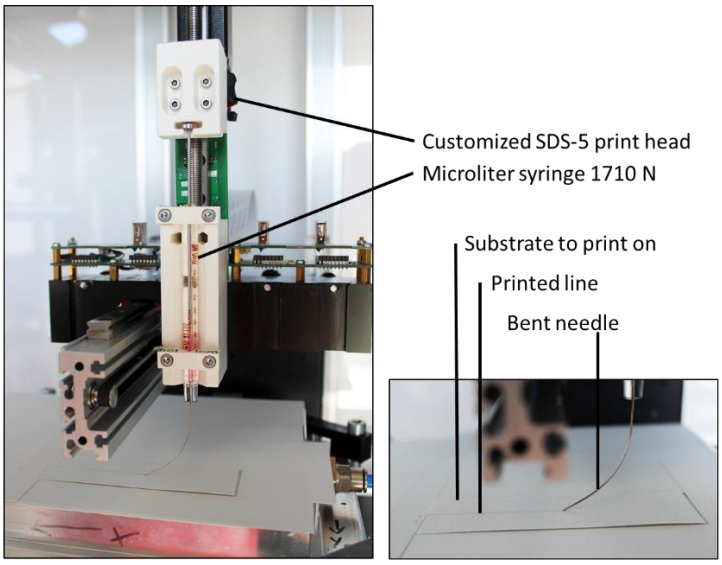


**Figure S8. Photography of the printing area of the 3D-printer System 30M used to strip the detection antibodies onto the membrane or paper.** The customizes SDS-5 print head loaded with the microliter syringe 1710 N including the bent needle can be seen. As a showcase red colored water (to see the printed line) is printed onto a paper cardboard (to distinguish the thicker substrate from the self-adhesive cards covering the vacuum table). The inset on the lower right shows a detailed photography of the print.

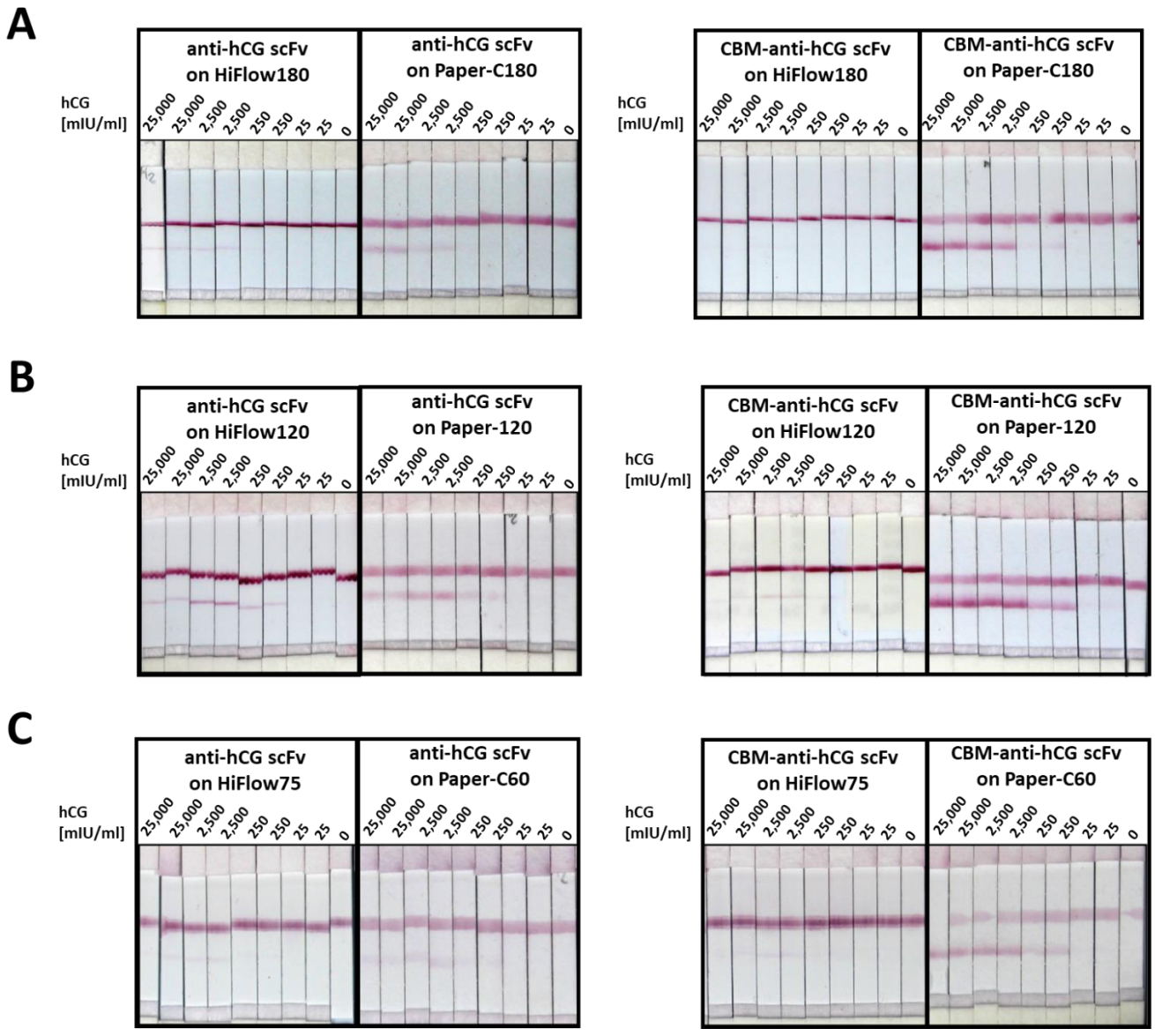


**Figure S9. LFTs for the detection of hCG using the CBM-anti-hCGH scFv or the anti-hCG scFv. Different cellulose-based papers (C180, C120, C60) and NC membranes (HiFlow180, HiFlow120, HiFlow75) were used.** (**A**) LFTs based on HiFlow180 nitrocellulose membranes or Paper-C180 (**B**) LFTs based on HiFlow120 nitrocellulose membranes or Paper-C120 (**C**) LFTs based on HiFlow75 nitrocellulose membranes or Paper-C60. 150 µl of sample was applied to the sample pad, containing 150 µl synthetic urine with varying concentrations of hCG. Sample compositions are listed in Table S1.

# Supplementary Tables

**Table S1. Sample composition for the validation of the pregnancy lateral flow assays.**

| Sample ID | Synthetic urine (hCG negative) [µL] | Human chorionic gonadotropin (hCG) [mIU/ml] | Human chorionic gonadotropin (hCG) [mIU] |
|---|---|---|---|
| **h1** | 150 | 25,000 | 3,750 |
| **h2** | 150 | 2,500 | 375 |
| **h3** | 150 | 250 | 37.5 |
| **h4** | 150 | 25 | 3.75 |
| **hCG negative control (hNC)** | 150 | 0 | 0 |

**Table S2. Sample composition for the validation of the Covid-19 antibody lateral flow assays.**

| Sample ID | anti-Sars-CoV-2 IgG [µg] | Human Serum (7.5 to 22 µg IgG/µl) [µl] | Total IgG [µg] | anti-Sars-CoV-2 IgG [%] |
|---|---|---|---|---|
| **s1** | 1 | 20 | (15 to 44)+1 | 2.22 to 6.25 |
| **s2** | 0.5 | 20 | (15 to 44)+0.5 | 1.11 to 3.32 |
| **s3** | 0.25 | 20 | (15 to 44)+0.25 | 0.56 to 1.63 |
| **s4** | 0.125 | 20 | (15 to 44)+0.125 | 0.28 to 0.82 |
| **s5** | 0.0625 | 20 | (15 to 44)+0.0625 | 0.14 to 0.41 |
| **s6** | 0.03125 | 20 | (15 to 44)+0.03125 | 0.07 to 0.2 |
| **negative control sample (sNC)** | 0 | 20 | 15 to 44 | 0 |
| **Positive control sample (sPC)** | 1 | 0 | 1 | 100 |

**Table S3. Protein Sequence information of CBM-scFv and full-length antibodies fused to CBM (IgG-CBM), including linker sequences and purification tag.**

| Protein | Sequence Information |
|---|---|
| **CBM-scFv** | ANTPVSGNLKVEFYNSNPSDTTNSINPQFKVTNTGSSAIDL<br>SKLTLRYYYTVDGQKDQTFWSDHAAIIGSNGSYNGITSNVK<br>GTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAK<br>NDWSNYTQSNDYSFKSASQFVEWDQVTAYLNGVLVWGK<br>EPGELGSVVPSTQPVTTPPATTKPPATTIPPSDDPNLEVLFQ<br>GPAS-scFv protein sequence-GSWSHPQFEK |
| **Full-length antibody-CBM** | Antibody heavy chain protein sequence-<br>GSGNATPTKGATPTNTATPTKSATATPTRPSVPTNTPTNTPA<br>NTPVSGNLKVEFYNSNPSDTTNSINPQFKVTNTGSSAIDLS<br>KLTLRYYYTVDGQKDQTFWSDHAAIIGSNGSYNGITSNVK<br>GTFVKMSSSTNNADTYLEISFTGGTLEPGAHVQIQGRFAK<br>NDWSNYTQSNDYSFKSASQFVEWDQVTAYLNGVLVWGK<br>EPGGSHHHHHH |