**Immunoglobulin single-chain variable fragment (scFv) generation**

The scFvs, provided by the Human Antibody Therapeutic Unit, SciLifeLab, Sweden, were constructed by cloning the VH and VL domains into an in‑house expression plasmid in the format VH-linker-VL-His tag, and transformed TOP10 cells were cultivated in 2XYT broth. Expressed scFvs were affinity purified on a cobalt matrix using gravity flow. The binding was confirmed by ELISA and SPR. The working concentration is 1 µg/mL of each antibody. In a preliminary experiment, ten scFvs were screened in pairs with twelve Fabs against serial dilution of recombinant Spike (RBD) and supernatant of SARS-Cov-2 infected Calu-3 cells (**Supplementaty Table 1** and **Supplementary Table 2**). The test was performed using a Luminex set up where the Fabs were coupled on beads as capture and the scFvs as detection. ScFvs showing the best performance as detection reagents were used for the following method development for dual detection.