Electronic Supplementary Material (ESI) for Sensors & Diagnostics.
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SUPPLEMENTARY SECTION

1.1 Optimization of the Capture Antibody Concentration

To determine the optimum concentration of the capture antibody, we coated the wells of a 96-well, F-bottom (Chimney Well) black, fluotrac, high-binding, sterile microtiter plate with 100 µL aliquots of varying concentrations of the capture antibody ranging from 0 to 5 µg/mL. Each concentration was diluted with coating buffer and were placed to representative wells in quadruplicates. The plate was sealed using a transparent ELISA sealing film (BrandTech-Thomas Scientific, Swedesboro, NJ) and incubated overnight at 2-8 °C. The next day, the plate was washed and an aliquot of 100 µL of 1 µg/mL of COVID-19 S1 spike Ag in COVID-19 incubation buffer was added into each well. The plate was then incubated at room temperature using a plate shaker at 500 rpm for 120 minutes. After incubation the plate was washed, and an aliquot of 100 µL of 1 µg/mL of primary antibody in COVID-19 incubation buffer each well was added with. The plate was incubated at room temperature using a plate shaker at 500 rpm for 120 minutes. Following incubation, the plate was washed and an aliquot of 100 µL of 1 µg/mL biotinylated secondary antibody in COVID-19 incubation buffer added with and incubated at room temperature using a plate shaker at 500 rpm for 90 minutes. After incubation, the plate was washed and 100 µL of 1 µM MOF-ZIF8 TA2-Gluc in COVID-19 incubation buffer was added followed by incubating at room temperature using a plate shaker at 500 rpm for 60 minutes. After final washing step, bioluminescent signal was measured by adding 100 µL of 5 µg/mL substrate coelenterazine

1.2 Generation of the Calibration Curve for the Spike Protein

A calibration curve for the spike protein was performed by coating a 96-well, F-Bottom (Chimney Well) black, Fluotrac, high-binding, sterile microtiter plate. An aliquot of 100 µL of 3 µg/mL capture antibody in coating buffer was placed into each representative well in quadruplicates. The plate was sealed using a transparent ELISA sealing film (BrandTech-Thomas Scientific, Swedesboro, NJ) and incubated overnight at 2-8 °C. The next day, the plate was washed using the wash buffer. Then aliquots of 100 µL of varying COVID-19 S1 spike antigen, concentrations ranging from 0.1 to 5 µg/mL in COVID-19 incubation buffer was added into each well. The plate was then incubated at room temperature using a plate shaker at 500 rpm for 120 minutes. After 120 minutes of incubation, the plate was washed, and aliquots of 100 µL of 2 µg/mL of primary antibody in COVID-19 incubation buffer was added into each well. The plate was incubated at room temperature using a plate shaker at 500 rpm for 120 minutes. After incubation, the plate was washed and 100 µL of 0.032 µg/mL biotinylated secondary antibody in COVID-19 Incubation Buffer was added and incubated at room temperature using a plate shaker at 500 rpm for 90 minutes. After incubation of the biotinylated secondary antibody, the plate was washed, and the wells were treated with 100 µL of 1 µM MOF-ZIF8 TA2-Gluc in COVID-19 incubation buffer and incubated at room temperature using a plate shaker at 500 rpm for 60 minutes. Finally, after incubation, a final washing was performed, and the plate was added with 100 µL of 5 µg/mL substrate coelenterazine to produce a bioluminescent signal. The light intensity of the produced bioluminescence was measured using a highly sensitive luminometer set at a fixed gain of 1700 and a time interval of 7.40 seconds.

1.3 Optimization of Primary and Secondary Antibody Concentration

To determine the optimum concentrations of the primary and biotinylated secondary antibody pairs, we performed a checkerboard assay (titration of primary and biotinylated secondary antibody in a microtiter plate). For this purpose, we coat a 96-well, F-Bottom (Chimney Well) Black, Fluotrac, High-Binding, Sterile microtiter plate with 100 µL of 3 µg/mL capture antibody in triplicates. The plate was sealed using a transparent ELISA sealing film (BrandTech-Thomas Scientific, Swedesboro, NJ) and incubated overnight at 2-8 °C. The next day, the plate was washed using the wash buffer. Aliquots of 100 µL of 0.2 µg/mL COVID-19 S1 spike antigen in COVID-19 incubation buffer were added into the odd-numbered wells (Columns 1, 3, 5...etc) while the even number wells (columns 2, 4, 6… etc.) were filled with 100 µL COVID-19 incubation buffer as blanks.

The plate was then incubated at room temperature using a plate shaker at 500 rpm for 120 minutes. After 120 minutes of incubation the plate was washed. 100 µL of the primary antibody was added in varying concentrations ranging from 1 to 4 µg/mL in COVID-19 incubation buffer across the rows and incubated at room temperature using a plate shaker at 500 rpm for 120 minutes. After incubation, the plate was washed and 100 µL of the biotinylated secondary antibody was added in varying concentrations ranging from 0.125 to 0.032 µg/mL in COVID-19 incubation buffer across the columns. The plate was incubated at room temperature using a plate shaker at 500 rpm for 90 minutes. After incubation of the biotinylated secondary antibody, the plate was washed and added with 100 µL of 1 µM MOF-ZIF8 TA2-Gluc in COVID-19 incubation buffer and incubated at room temperature using a plate shaker at 500 rpm for 60 minutes. After incubation, final washing was done, and the plate was added with 100 µL of 5 µg/mL substrate coelenterazine to produce a bioluminescent signal. The light intensity of the produced bioluminescence was measured using a highly sensitive luminometer set at a fixed gain of 1700 and a time interval of 7.40 seconds.

1.4 Optimization of the Incubation Time for the COVID-19 S1 Spike Antigen

The optimized incubation time for the COVID-19 spike antigen was performed by using the procedure described in 1.2 except during the incubation of the spike antigen, the time for incubation was varied between 15 to 180 minutes for each replicate.

1.5 Optimization of the Incubation Time for the Primary and Biotinylated Secondary Antibody

The optimized incubation time for the primary and secondary antibodies were performed by using the procedure described in 1.2 except during the incubation of the primary and secondary antibodies, the time for incubation for both antibodies were between 1 minute to 3 hours on a 30-minute interval for each replicate.

1.6 Optimization of the Incubation Time for TA2-Gluc

The optimized incubation time for TA2-Gluc was performed by using the procedure described in 1.2 except during the incubation of TA2-Gluc, the time for incubation was between 1 minute to an hour for each replicate.