ELISPOT

A peptide pool covering the whole S protein sequence (15-mer sequences with 11aa overlap) is prepared in solutions at two times final concentration and added to a 96 well plate, 100µl/well. PBMC concentration is adjusted to desired concentration, e.g.: 3 million/ml corresponding to 300,000 cells/well) and plated, 100µl/well using large orifice tips. The plates are incubated for 48-72 hours in a 37°C humidified incubator, 9% CO_2.

Plates are washed three times with 0.05% Tween-PBS, 200µl/well and anti-human IFN-γ/IL-5 Detection Solution is added (80µl/well). Plates are incubated at room temperature, two hours.

Plates are washed three times with 0.05% Tween-PBS, 200µl/well and the Tertiary Solution is added (80µl/well). Plates are incubated at room temperature, two hours.

Plates are washed two times with 0.05% Tween-PBS, and then two times with distilled water, 200µl/well each time before adding the Developer Solution (IFNγ), 80µl/well. Plates are incubated at room temperature, 15 minutes. The same steps are repeated for the second Developer Solution (IL-5).

Plates are air-dried for two hours in running laminar flow hood or for 24 hours face down on paper towels on bench top and then scanned and counted with an Immunospot analyzer.