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1.0 Introduction

1.1 CSA: SARS-CoV-2 Overview

The health and well-being of your non-human primate (NHP) colonies are critical to the success of your research. The Colony Surveillance Assay™: SARS-CoV-2 kit provides results that indicate exposure to SARS-CoV-2 by identifying specific immunoglobulins in serum.

The CSA: SARS-CoV-2 kit is intended to be used to identify naïve animals prior to studies that could be affected by previous immune response to SARS-CoV-2 or other seasonal coronaviruses. Kits can also be used to perform serial dilutions of serum to titer antibody against SARS-CoV-2 antigens or to screen individuals for seropositivity.

The CSA: SARS-CoV-2 kit consists of reagents sufficient to process up to ninety-four samples and the included control sera. The CSA: SARS-CoV-2 kit uses the same basic laboratory instruments as an ELISA. The SilverQuant® chromogenic reagents are used for signal generation on the CSA array products. Assay results are measured using the AQ 1000 scanner, where data is easily generated using a scanner and quickly analyzed using the included software.

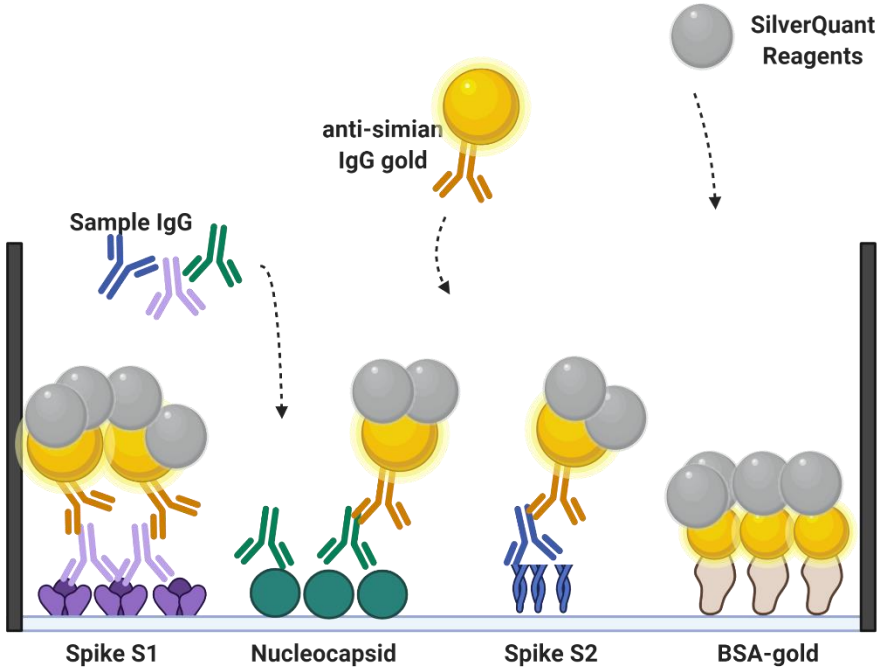

Figure 1. CSA: SARS CoV-2 Assay Diagram. In each of the 96 wells of the array plates, antigens are immobilized to the surface and blocked. When sample is added to a well, any antigen-specific IgG will bind to the antigen. Unbound material is washed away. Immune complexes are visualized with the Gold Conjugate and SilverQuant reagents. Created with BioRender.com

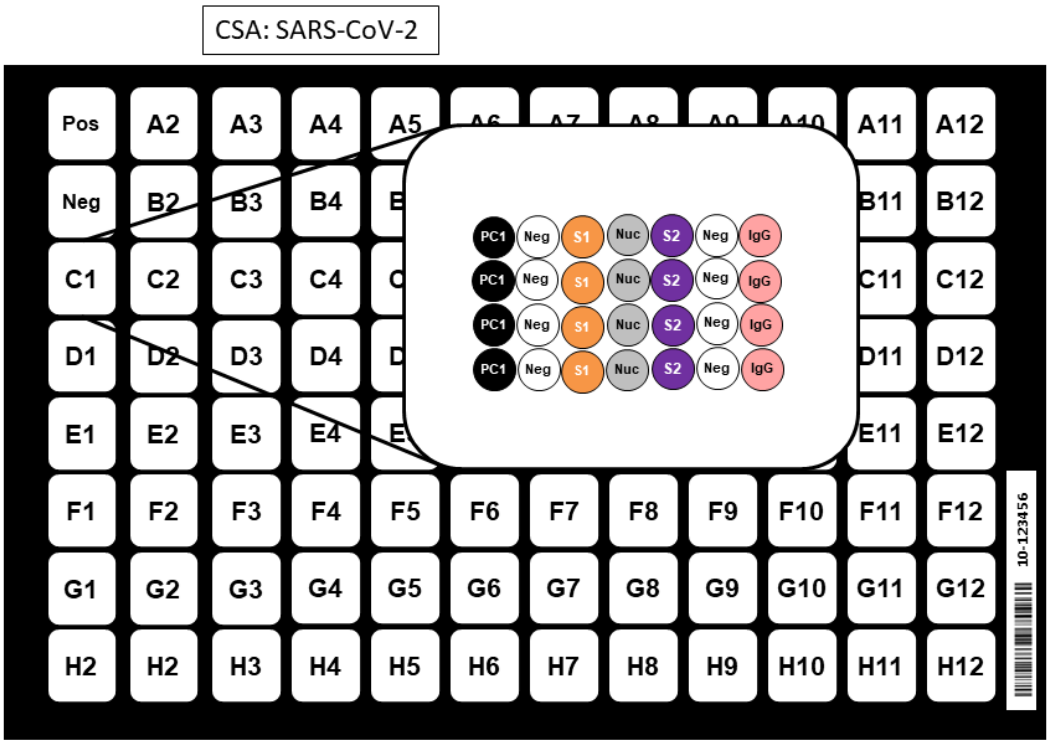

Figure 2. CSA: CoV-2 Well Diagram. Each of the 96 wells of the array plates contains 4 replicate spots for the Spike S1 domain, the Spike S2 domain, and the Nucleoprotein as shown. Positive Control (PC1 and IgG) and Negative Control spots are also printed with 4 replicates in each well. Each well contains the same immobilized antigens and controls in the grid pattern.

2.0 Kit Contents

Component	Description	Quantity	Prod. No.
CSA: SARS-CoV-2 Plate	One 96-well array plate containing 3 printed antigens representing 2 SARS-CoV-2 proteins	1 plate	12-1221
5X Slide Wash Buffer	Buffer used to remove unbound protein	100 mL	2-1039
CSA Buffer	Buffer used to dilute samples and Gold Detection Reagent	100 mL	7-1037
SARS-CoV-2 Positive Control	Positive control sample	10 µL	12-1071
Negative Control	Negative control sample	10 µL	12-1009
Anti-simian Gold Conjugate	Gold Detection Reagent	100 µL	10-2139
SilverQuant Reagent A	Development Reagent A	10 mL	10-2132
SilverQuant Reagent B	Development Reagent B	10 mL	10-2112
Plate Well Seal	Used to seal wells during sample incubation	1 each	4-1009
CSA: SARS-CoV-2 Kit User Protocol			L792

3.0 Required Materials and Equipment

Component	Description
Bench-top microcentrifuge	Capacity to hold 1.5 mL microcentrifuge tubes
Vortex mixer	Various
96-well dilution plate	96 well plate capable of holding 0.5 mL per well
50 mL conical tubes	Various
Deionized or ultrapure water	Clean water
Microarray scanner	AQ 1000
Microarray image analysis software	AQ Pro software (included with Product AQ 1000)
12-1221 CSA: SARS CoV-2 data analysis template	Included with AQ 1000 scanner
Micropipettes	Single channel capable of 5 µL, 80 µL, and 8-channel (1-300 µL)
Serologic pipettor and disposable pipettes	Capable of pipetting 10-25 mL
Reagent reservoirs	Capable of holding at 25-100 mL

4.0 Storage

The CSA: SARS-CoV-2 Kit (Prod. No. 12-1225) should be stored at 2-8˚C until used.

5.0 Safety and Handling

Use Universal Safety Precautions when handling animal body fluids. For all other materials, normal precautions exercised in handling laboratory materials should be followed. The material is not considered hazardous according to 29CFR1910.1200. The chemical, physical, and toxicological properties of this product may not, as yet, have been thoroughly investigated. We recommend the use of gloves, lab coats, and eye protection when working with any material.

6.0 Protocol Overview

The CSA: SARS-CoV-2 kit contains sufficient reagents for qualitative analysis of up to 94 serum samples if run as a complete kit. However, only a single assay may be performed with the reagents included in the kit. Reagent B is air sensitive and must be used within one day of opening. Both Reagent A and Reagent B are light sensitive and should not be exposed to direct or excess light.

The assay workflow begins with preparation of the sample dilutions. Once samples and controls are diluted in the provided dilution plate, the location of each sample and control is recorded. Samples are added to the assay plate for an hour incubation, then rinsed with Wash Buffer and the Anti-simian Gold Conjugate is added. After an hour incubation, the conjugate is removed, and the plate is washed. Lastly, the SilverQuant Reagent A and Reagent B are mixed, quickly added to each well, and incubated in the dark for 3 minutes.

If desired, partial plates can be run, but fresh Reagent A and Reagent B must be used. Additional reagents for regular use of partial plates are available with Product No. 10-2132 and 10-2112.

CSA: SARS-CoV-2 plates should be handled with care. Never touch the interior bottom of the well, and do not allow them to dry during the assay once they have been wetted.

Proper storage and handling of serum samples is critical for obtaining optimal data. Avoid repeated freeze-thaw cycles and aliquot and freeze samples at -80°C for long-term storage.

Developed CSA array plates are stable and can be stored indefinitely at room temperature.

7.0 Procedure

7.1 Preparation of Buffers and Reagents

7.1.1 Remove a CSA: SARS-CoV-2 Kit (12-1225) from refrigerator, open the bag, and remove the kit contents.

7.1.2 Return the following items to the refrigerator until needed: (1) Anti-simian Gold Conjugate (10-2139), (2) SilverQuant Reagent A (10-2132), and (3) SilverQuant Reagent B (10-2112).

7.1.3 In a container capable of holding at least 500 mL, add 400 mL of deionized or ultrapure water. Add 100 mL of the 5X Slide Wash Buffer (2-1039). Mix thoroughly. Store closed at room temperature for up to 1 month. Label as “1X Slide Wash Buffer”.

7.1.4 Allow the CSA: SARS-CoV-2 Plate (12-1221) and the CSA Buffer (7-1037) to warm to room temperature before starting the assay.

7.2 Serum Dilution and Addition to the Array

NOTE: If using partial plate, make sure the unused wells will not be wetted at any time and can be used in the future. A plate seal can be used for this purpose.

NOTE: After step 7.2.3, do not allow the surface of the array to dry completely at any time before you are ready to scan the plate.

7.2.1 Note the location of each sample to be loaded in the plate map below. **The Positive and Negative Controls must be run with <u>each use</u> of the assay plate.**

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive Control											
B	Negative Control											
C												
D												
E												
F												
G												
H												

7.2.2 Ensure that each sample is completely thawed and vortex 3-5 seconds to mix. Spin each sample at 5,000 rpm for at least 5 seconds to collect the material in the bottom of the tube.

7.2.3 To the 96-well dilution plate (not included with kit), pipette 500 µL of CSA Buffer (7-1037) into all wells.

7.2.4 Add 5 µL Negative Control (12-1009) to well B1 of the 96-well dilution plate.

7.2.5 Add 5 µL of each serum sample to be tested to remaining wells of the 96-well dilution plate as indicated in the table above.

7.2.6 Add 5 µL of SARS-CoV-2 Positive Control (12-1071) to well A1 of the 96-well dilution plate.

NOTE: If your serum sample has been diluted, please adjust the dilution accordingly for a 1:100 dilution into the well dilution plate.

7.2.7 Set multichannel pipette to 150 µL. For each column in the dilution plate, mix each sample by pipetting up and down 3 times prior to drawing up 150 µL.

7.2.8 Dispense 150 µL of diluted sample into each well of the CSA: SARS-CoV-2 Plate (12-1221), taking care not to touch the bottom of the well.

7.2.9 Cover the wells with the provided Plate Well Seal (4-1009) to prevent evaporation. Tap the side of the plate and incubate at room temperature for **1 hour**.

7.2.10 Discard sample dilution plate into appropriate biohazard waster container.

7.3 Wash 1 and Add Gold Conjugate Reagent

7.3.1 About 5-10 minutes before the sample incubation (step 7.2.9) is complete, remove a tube of Anti-simian Gold Conjugate (10-2139) from the refrigerator.

7.3.2 Briefly spin the tube of Anti-simian Gold Conjugate (10-2139) using a bench top microcentrifuge to collect all the material into the bottom of the tube.

7.3.3 Prepare the Gold Conjugate Reagent by adding 18 mL of CSA Buffer (7-1037) to a new 50 mL conical tube. Pipette **90 µL** of Anti-simian Gold Conjugate (10-2139) into the middle of the liquid, taking care not to pipette on the side of the tube. Cap the tube and mix by gently inverting 3-5 times, taking care not to create bubbles in the tube.

NOTE: If using less than a full plate, adjust dilution volume accordingly. Final dilution of Anti-simian Gold is 1:200. Calculate total volume needed for using 150 µL per well, plus overage for using with a multichannel pipette.

7.3.4 When the 1 hour incubation (**Step 7.2.9**) is complete, remove the serum solutions from wells by firmly shaking the inverted plate over a biohazardous waste container until all material has been removed. Wipe top of plate with a lint-free wipe to remove any excess material.

7.3.5 Add 150 µL of 1X Slide Wash Buffer (prepared in **Step 7.1**) to each well using a multi-channel pipettor and tap side of the plate. Remove Slide Wash Buffer by inverting plate over a liquid biohazard waste container.

7.3.6 Repeat step 7.3.5 two more times, adding 150 µL of Slide Wash Buffer for a total of 3 washes in the plate.

7.3.7 Remove the final wash and immediately add 150 µL Gold Conjugate Reagent (prepared in **Step 7.3.3**). Tap side of the plate to ensure bottom of the wells are completely covered.

7.3.8 Incubate for **1 hour** at room temperature. About halfway through the incubation period, remove SilverQuant Reagent A (10-2132) and SilverQuant Reagent B (10-2112) from the refrigerator and allow to come to room temperature.

7.4 Wash 2 and Development

7.4.1 Remove the Gold Conjugate Reagent from the plate by inverting over a waste container. Shake plate firmly to remove liquid from bottom of wells.

7.4.2 Add 150 µL of 1X Slide Wash Buffer to each well using a repeat or multi-channel pipettor. Tap side of plate. Remove Slide Wash Buffer by inverting plate over liquid waste.

7.4.3 Repeat **Step 7.4.2** two times, for a total of three washes. After final wash keep Slide Wash Buffer into wells until ready to perform **step 7.4.7.**

SilverQuant Reagent A (Product No. 10-2132) and SilverQuant Reagent B (Product No. 10-2112) are sensitive to light. Be sure to perform the following steps out of direct sunlight.

It is CRITICAL to add the reagent mix quickly to the CSA: SARS-CoV-2 (12-1221) plate because the reaction is time dependent. Once Reagent A and B have been mixed together and added to the reagent trough, quickly add to the plate using a multi-channel pipettor.

Be sure to read and understand **Steps 7.4.4 - 7.5.1** and have all the needed equipment prepared and ready.

7.4.4 Set a timer to 3 minutes.

7.4.5 Add pipet tips to a multichannel pipettor and set to **100 µL**. Obtain a fresh reagent trough capable of holding 20 mL of liquid.

7.4.6 Remove Slide Wash Buffer from the wells by inverting plate over liquid waste. Dry plate with lint-free wipe.

7.4.7 Prepare the SilverQuant Development Reagent by directly pouring SilverQuant Reagent A (10-2132) into SilverQuant Reagent B bottle (10-2112). Cap the bottle, shake for 3 seconds, and pour into reagent trough.

7.4.8 Immediately add 100 µL of the SilverQuant Development Reagent to each well of the CSA: SARS-CoV-2 plate (12-1221) using the multichannel pipette. Tap the side of the plate twice to ensure that the development reagent covers the entire bottom of the plate.

7.4.9 Immediately start the timer and incubate for <u>exactly</u> 3 minutes. Place a cover (i.e. the lid of a box) over the plate to protect it from light.

7.4.10 Obtain a squirt bottle filled with fresh ultra-pure water and place next to liquid waste container.

7.5 Final Rinse

7.5.1 When the incubation time expires, invert the CSA: SARS-CoV-2 Plate (12-1221) to remove the development reagent from the plate into a proper chemical waste container and immediately fill the wells with ultrapure water using the squirt bottle. Remove water by inverting plate over sink or liquid waste.

7.5.2 Repeat the water flush to ensure all the development reagent is rinsed out.

7.5.3 Dry the plate by shaking vigorously to remove liquid and tapping onto absorbent towels. Alternatively, the plate can be dried by using a plate centrifuge. Invert the plate over an absorbent towel and gently spin down the plate (3,000 rpm for 1 minute).

7.5.4 Empty any unused SilverQuant Development Reagent into a chemical waste container.

7.5.5 Scan and analyze the CSA: SARS-CoV-2 plate using the template designated in the Certificate of Analysis provided inside the kit.

8.0 Ordering Information

Telephone: +1.608.561.8730

Email: orders@intuitivebio.com or support@intuitivebio.com

Website: www.intuitivebio.com

9.0 Appendix A: Troubleshooting

Problem: Weak Signal

Suggested Causes & Solutions:

1. Incorrect assay temperature – Reaction must occur at 18- 30°C for optimal results.
2. Protein degradation – Use freshly prepared samples.
3. Slow addition of the development reagent – Mix Reagent A and B quickly and immediately add the development reagent to plate without hesitation. Use a multichannel pipette to ensure rapid delivery of reagent to each well.
4. Incorrect assay incubation time – Follow protocol for proper incubation times.
5. If BSA-Gold signal is low, check SilverQuant Reagents to ensure proper temperature at use.
6. If IgG signal is low, check that the Anti-simian Gold Conjugate was diluted and added properly.

Problem: High Background

Suggested Causes & Solutions:

1. SilverQuant chromogenic reagents were exposed to light for an extended period – SilverQuant Reagents A and B should have minimal exposure to direct light. Seal the plate immediately after the development reagent is added to the wells.
2. Incomplete washing – Wash as directed.

Problem: No signal from IgG controls

Suggested Causes & Solutions:

1. A step in the protocol was skipped or a reagent was mishandled – Follow protocol carefully.
2. A low signal is seen in the IgG controls – the anti-simian IgG gold conjugate addition was skipped or the preparation was mishandled.
3. Repeat with a fresh kit.

Problem: Heterogeneous Background

Suggested Causes & Solutions:

1. Washing/Drying artifact – Salts in the wash buffer may leave "streaks" in the array image. Briefly rinse wells again with purified water and immediately dry.
2. Dust may adhere to the well after it has been dried. It may be necessary blow/wipe off any dust that may have settled into a well rinse with water and dry.
3. High background samples – Some samples result in a high background signal in the areas surrounding the spots in the array. If a repeat assay results in a second high background test, redraw the blood sample and process fresh serum.