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		Revision A	DCO # 25005

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1.0 Introduction

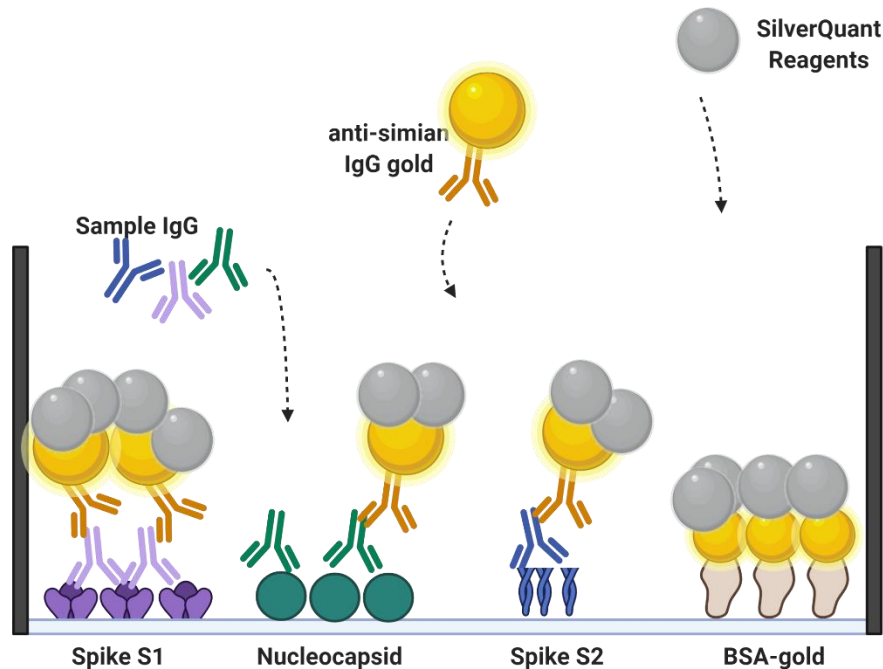
1.1 CSA: Simian SARS-CoV-2 Overview

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 IAN scanner, where data is easily generated using a scanner and quickly analyzed using the included software.

Figure 1. CSA: Simian SARS-CoV-2 Schematic



1.2 SilverQuant Surface Chemistry

The CSA: SARS-CoV-2 arrays are printed on Intuitive Biosciences' proprietary protein plates and are specifically designed for multiplex immunoassays and deliver high signal-to-noise with high sensitivity for protein microarray applications.

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2.0 Kit Contents

Component	Description	Prod. No.
CSA: Simian SARS-CoV-2 Plate	Plate containing 96 wells containing three printed antigens representing two SARS-CoV-2 proteins	12-1221
5X Plate Wash Buffer	Buffer used to remove unbound protein	2-1039
CSA Buffer	Buffer used to dilute samples and Gold Detection Reagent	7-1037
SARS-CoV-2 Positive Control	Positive control sample	12-1071
Negative Control	Negative control sample	12-1009
SilverQuant Anti-simian IgG Gold Conjugate	Gold Detection Reagent	10-2139
SilverQuant Reagent A	Development Reagent A	10-2132
SilverQuant Reagent B	Development Reagent B	10-2112
Well Seals	Used to seal wells during sample incubation	4-1009
96 Deep Well Dilution Plate	Used for serum dilutions	12-1025

3.0 Required Materials


Component	Description
Bench-top microcentrifuge	Capacity to hold 1.5 mL microcentrifuge tubes
Vortex Mixer	Various
1.5 mL Microcentrifuge tubes	Various
50 mL conical tubes	Various
Deionized or Ultrapure Water	Clean water
Microarray Scanner	Plate Scanner
Microarray Image Analysis Software	Plate reading computer software
CSA: Simian SARS-CoV-2 data analysis template	Included with reader software
Micropipettes	Single, Repeat, and 8-channel; various capacities

4.0 Storage

The CSA: Simian SARS-CoV-2 (Prod. No. 12-1225) kit 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.

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6.0 Protocol Overview

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

If desired partial plates can be run, but unopened Reagent A and Reagent B must be used. Additional reagents for regular use of partial plates are available. Do not mix reagents from different lots of kits. Only use the reagents that are provided within the kit.

CSA: Simian SARS-CoV-2 plates should be handled with care (never touch the bottom of the well) and not allowed to dry 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.

7.0 Procedure

7.1 Preparation of Buffers and Reagents


NOTE: Equilibrate entire kit to room temperature (18-30°C) prior to use for peak assay performance.

- 7.1.1 In a container capable of holding at least 500 mL, add 400 mL of ultrapure water. Add 100 mL of the 5X Plate Wash Buffer (Product No. 2-1039). Mix thoroughly. Store closed at room temperature for up to 1 month. Label as "1X Plate Wash Buffer".

7.2 Serum Dilution and Addition to the Array

NOTE: If using partial plate, cover the unused wells with plate seal (Prod. No. 4-1009) to make sure the wells will not be wetted at any time and can be used in the future.

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.

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7.2.1 Note the location of each sample to be loaded in the plate map below.

	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 briefly. Spin each sample at 5,000 rpm for at least 10 seconds to collect the material in the bottom of the tube.

7.2.3 To the 96 Well Dilution Plate (Product No. 12-1025), pipette 500 µL of CSA Buffer into all wells.

7.2.4 Add 5 µL of Positive Control serum (12-1071) to well A1 (or the Positive Control well) of the 96-well dilution plate.

7.2.5 Add 5 µL Negative Control (12-1009) to wells B1 (or the Negative Control well) of the 96-well dilution.

7.2.6 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.


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 5 times prior to drawing up 150 µL.

7.2.8 Dispense 150 µL of diluted sample into each well of the CSA: Simian SARS-CoV-2 Plate (Prod. No. 12-1221), taking care not to touch the bottom of the well (pipette into a corner or side of the well).

7.2.9 Cover the wells with the provided plate seal (Prod. No. 4-1009) to prevent evaporation, tap side of the plate and incubate at room temperature for 1 hour.

7.2.10 Discard sample dilution plate into appropriate biohazard waste container.

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7.3 Wash 1 and Add Gold Conjugate Reagent

7.3.1 Briefly spin the SilverQuant Anti-Simian IgG Gold Conjugate (Product No. 10-2139) using a bench top microcentrifuge to collect all the material into the bottom of the tube and gently vortex to mix.

7.3.2 Prepare the Gold Conjugate Reagent by adding 85 μ L of SilverQuant Anti-Simian IgG Gold Conjugate to 17 mL of CSA Buffer. Mix gently and thoroughly.

NOTE: If using less than a full plate, adjust dilution volume accordingly. Final dilution of Anti-Simian IgG Gold is 1:200.

7.3.3 Remove the serum solutions from wells by covering the CSA: Simian SARS-CoV-2 Plate with a paper towel or other absorbent paper, invert, and, while holding the paper towel, tap down three times. Dispose of the adsorbent paper into a biohazardous waste container.

7.3.4 Add 150 μ L of 1X Plate Wash Buffer (PWB, prepared in **Step 7.1.1**) to each well using a repeat or multi-channel pipettor and tap side of the plate. Remove PWB by inverting plate over a liquid biohazard waste container.

7.3.5 Repeat step 7.3.4 two more times, adding 150 μ L of 1X Plate Wash Buffer for a total of 3 washes in the Plate.

7.3.6 Remove the final wash and tap the plate on a paper towel to remove all wash buffer from the wells. Immediately add 150 μ L Gold Conjugate Reagent (prepared in **Step 7.3.2**). Tap side of the plate to ensure bottom of the wells are completely covered.

7.3.7 Incubate for **1 hour** at room temperature.

7.4 Wash 2 and Development

7.4.1 Remove the Gold Conjugate Reagent from the CSA: Simian SARS-CoV-2 Plate inverting over a waste container. Shake plate firmly to remove liquid from bottom of wells.

7.4.2 Add 150 μ L of 1X Plate Wash Buffer to each well using a repeat or multi-channel pipettor. Tap side of plate. Remove Plate 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 Plate Wash Buffer into wells until ready to perform **step 7.4.7**.


NOTE: 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 **CRITICAL** to add the reagent mix quickly to the CSA: Simian SARS-CoV-2 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 plate using a multi-channel pipettor or repeat 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 pipette tips to a multichannel and prepare it to dispense **100 μ L**. Obtain a fresh reagent trough capable of holding 20 mL of liquid.

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- 7.4.6 Prepare the SilverQuant Development Reagent by directly pouring SilverQuant Reagent A (Product No. 10-2132) into SilverQuant Reagent B bottle (Product No. 10-2112). Cap the bottle and shake vigorously for ~3 seconds and pour into reagent trough.
- 7.4.7 Quickly remove Plate Wash Buffer from the wells by inverting plate over liquid waste and tap the plate on a paper towel to remove all wash buffer from the wells. Immediately add **100 µL** of the SilverQuant Development Reagent to each well of the CSA: Simian SARS-CoV-2 Plate using the multichannel repeater pipette. Tap the side of the plate twice to ensure that the Development Reagent covers the entire bottom of each well of the plate.
- 7.4.8 Immediately start the timer and incubate for exactly 3 minutes. Place a cover (i.e. the lid of a box) over the CSA: Simian SARS-CoV-2 Plate to protect it from light.
- 7.4.9 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: Simian SARS-CoV-2 Plate 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 twice to ensure all the Development Reagent is rinsed out.
- 7.5.3 Dry the CSA: Simian SARS-CoV-2 Plate by either using a plate centrifuge (inverting plate over paper towel and gently spin down the plate) or by letting air dry on the benchtop.
- 7.5.4 Empty any unused Development Reagent into a chemical waste container.
- 7.5.5 Scan and analyze the CSA: Simian SARS-CoV-2 Plate using the template designated in the Certificate of Analysis from the kit.

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8.0 Ordering Information

Telephone: +1.608.826.4240

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 – immediately add the Development Reagent to reaction tube, and cap without hesitation.
4. Incorrect assay incubation time – follow protocol for proper incubation times.

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.

Problem: No signal from detection controls

Suggested Causes & Solutions:

1. A step in the protocol was skipped or a reagent was mishandled.
2. If a low signal is seen in the positive controls, the anti-simian IgG gold conjugate solution was possibly missing or mishandled.

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.

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Intuitive Biosciences' thin nitrocellulose protein microarray plate is covered by several US and foreign patents, including US Patent #6,861,251 and #7,235,307.

Other US and international patents pending.

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