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the variability in final use conditions and material handling by the user. No claims beyond replacement of unacceptable material or refund of purchase price shall be allowed.

1.0 Introduction

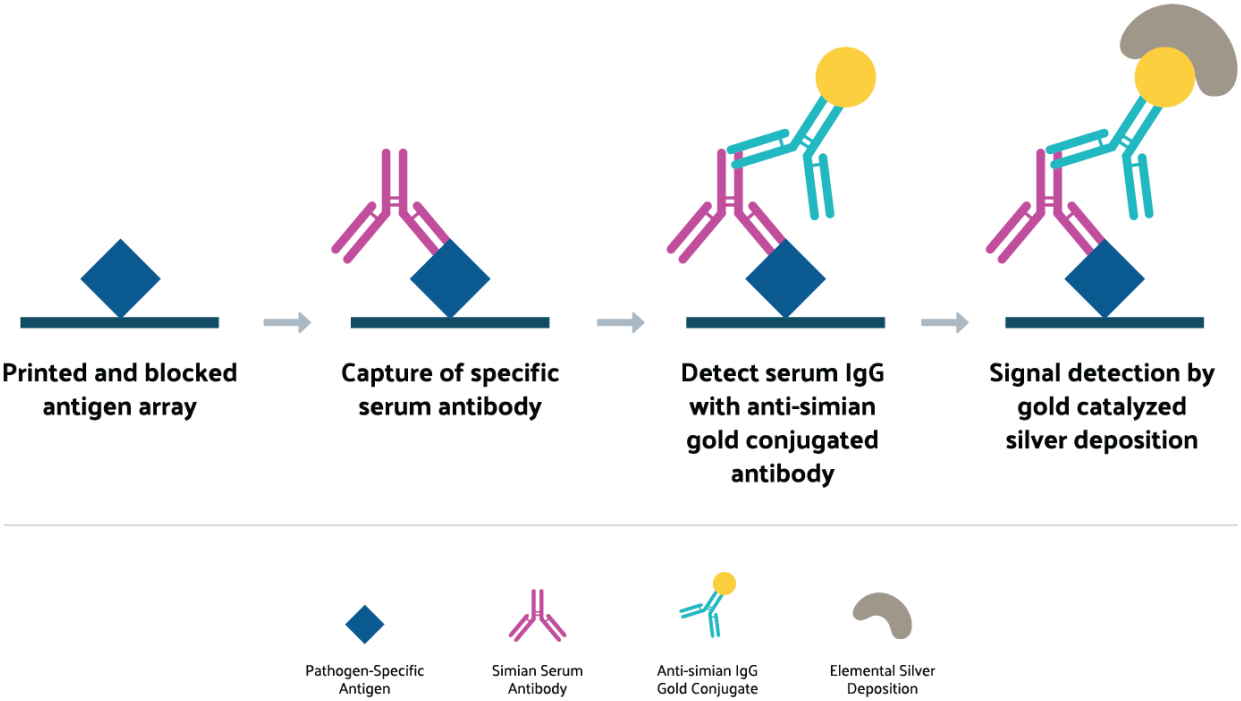
1.1 CSA: Simian TB Overview

The health and well-being of your non-human primate (NHP) colonies are critical to the success of your research. The Colony Surveillance Assay™: Simian TB kit results can indicate previous exposure to *Mycobacterium tuberculosis* by identifying *M. tuberculosis* specific immunoglobulins in test serum.

The CSA: Simian TB kit is intended to be used in addition to the required tuberculin skin test (TST). The CSA: Simian TB assay provides a laboratory based test for identifying animals that have been exposed to or may be infected with *M. tuberculosis*. The results from this assay should be considered with results from other test methods, including the TST. Each institution should determine how to incorporate the results into their current *M. tuberculosis* screening protocols.

The CSA: Simian TB kit consists of reagents sufficient to process up to ninety-two samples, and the included control sera. The CSA: Simian TB kit uses the same basic laboratory instruments as an ELISA. The SilverQuant® chromogenic reagents are used for signal generation on the CSA: Simian 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: Simian TB Schematic



2.0 Kit Contents

Component	Description	Quantity	Prod. No.
CSA: Simian TB Plate	Plate containing 96 wells consisting of antigens representing epitopes from *M. tuberculosis*	1 plate	12-1214
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
Positive Control	Positive control sample	2 x 10 µL	12-1053
Negative Control	Negative control sample	10 µL	12-1009
SilverQuant Anti-simian IgG 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 Seals	Used to seal wells during sample incubation	1 each	4-1009
96 Deep Well Dilution Plate	Used for serum dilutions	1 each	12-1025
CSA: Simian TB Plate User Protocol			L762

3.0 Required Materials

Component	Description
Bench-top microcentrifuge	Capacity to hold 1.5 mL microcentrifuge tubes
Centrifuge with MTP rotor and plate racks	For drying plats with tabletop centrifuge using microtiter plate racks @ 3 minute low g-force spin. Optional step.
Vortex Mixer	Various
1.5 mL Microcentrifuge tubes	Various
15 mL conical tubes	Various
50 mL conical tubes	Various
Deionized or Ultrapure Water	Clean water
Microarray Scanner	AQ 1000
Microarray Image Analysis Software	AthenaQuant software (included with Product AQ 1000)
CSA: Simian TB data analysis template	Included with AQ 1000 scanner
Micropipettes	Single, Repeat, and 8-channel; various capacities

4.0 Storage

The CSA: Simian TB Kit (Prod. No. 12-1215) 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: Simian TB 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 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.

The assay workflow begins with preparing the sample dilutions and wetting the assay plate. Once samples and controls are diluted in the provided dilution plate, be sure to record the location of each sample and control. Samples are added to assay plate for 1 hour incubation, then rinsed with Wash Buffer and the anti-simian IgG gold conjugate is added. After 1 hour incubation, the conjugate is removed and the plate washed. Last, the SilverQuant A and B reagents are mixed and 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: Simian TB plates should be handled with care (never touch the bottom of the well) and not allowed 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

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 Slide Wash Buffer (Product No. 2-1039). Mix thoroughly. Store closed at room temperature for up to 1 month. Label as "1X Slide 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.

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 each use 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 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 Add 200 µL of CSA Buffer (Product No. 7-1037) to each well of the CSA:Simian TB Plate (Product No. 12-1214) using a multi-channel pipettor. Tap the side of the Plate unit two times to ensure that the buffer covers each well completely.

7.2.4 Immediately remove the CSA Buffer from the wells by inverting over a sink or liquid disposal container three times.

7.2.5 Using a multi-channel pipettor, immediately add 75 µL of fresh CSA Buffer to each well, tap side of the Plate and set aside. Remove excess solution from the top of the Plate by blotting dry with a lint-free wipe.

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

7.2.7 Add 10 µL of Positive Control serum (12-1053) to wells A1 of the 96-well dilution plate. Mix by pipetting up and down 3 times.

7.2.8 OPTIONAL: If running partial plates, you may need to conserve the amount of 12-1053 TB Positive Control serum used. Instead of adding 500 µL of CSA Buffer to A1 or other designated TB Positive control well, add 250 µL of CSA Buffer and 5 µL of 12-1053 TB Positive Control Serum.

7.2.9 Add 5 µL Negative Control (12-1009) to wells B1 of the 96-well dilution. Mix by pipetting up and down 3 times.

7.2.10 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. Mix by pipetting up and down 3 times.

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

7.2.11 Set multichannel pipette to 25 µL. For each column in the dilution plate, mix each sample by pipetting up and down 5 times prior to drawing up 25 µL.

7.2.12 Dispense 25 µL of diluted sample into each well of the CSA: Simian TB Plate (Prod. No. 12-1214), taking care not to touch the bottom of the well (pipette into a corner or side of the well).

7.2.13 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**.

7.2.14 Discard sample dilution plate into appropriate biohazard waster container.

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 55 µL of SilverQuant Anti-Simian IgG Gold Conjugate to 11 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. Calculate total volume needed for using 100 µL per well, plus overage for using a multichannel pipette.

7.3.3 Remove the serum solutions from wells by covering the CSA: Simian TB 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 200 µL of 1X Slide Wash Buffer (SWB, prepared in **Step 7.1.1**) to each well using a repeat or multi-channel pipettor and tap side of the plate. Remove SWB by inverting plate over a liquid biohazard waste container.

7.3.5 Repeat step 7.3.4 two more times, adding 200 µL of 1X Slide Wash Buffer for a total of 3 washes in the Plate.

7.3.6 Remove the final wash and immediately add 100 µL 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 TB Plate inverting over a waste container. Shake plate firmly to remove liquid from bottom of wells.

7.4.2 Add 200 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.**

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 TB Plate because the reaction is time dependent. Once Reagent A and B have been mixed together and added to the reagent trough, quick 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 pipet tips to a multichannel and prepare it to dispense 100 µL. Obtain a fresh reagent trough capable of holding 20 mL of liquid.

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 Slide Wash Buffer from the wells by inverting Plate over liquid waste. Dry plate with lint free wipe. Immediately add 100 µL of the SilverQuant Development Reagent to each well of the CSA: Simian TB Plate using the multichannel repeater pipette. Tap the side of the plate twice to ensure that the Development Reagent covers the entire bottom 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 TB 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 TB 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 TB Plate by either using a plate centrifuge (inverting plate over paper towel and gently spin down the plate) or let air dry after shaking vigorously to remove liquid.

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

7.5.5 Scan and analyze the CSA: Simian TB 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 – 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 or the scanner's glass surface.