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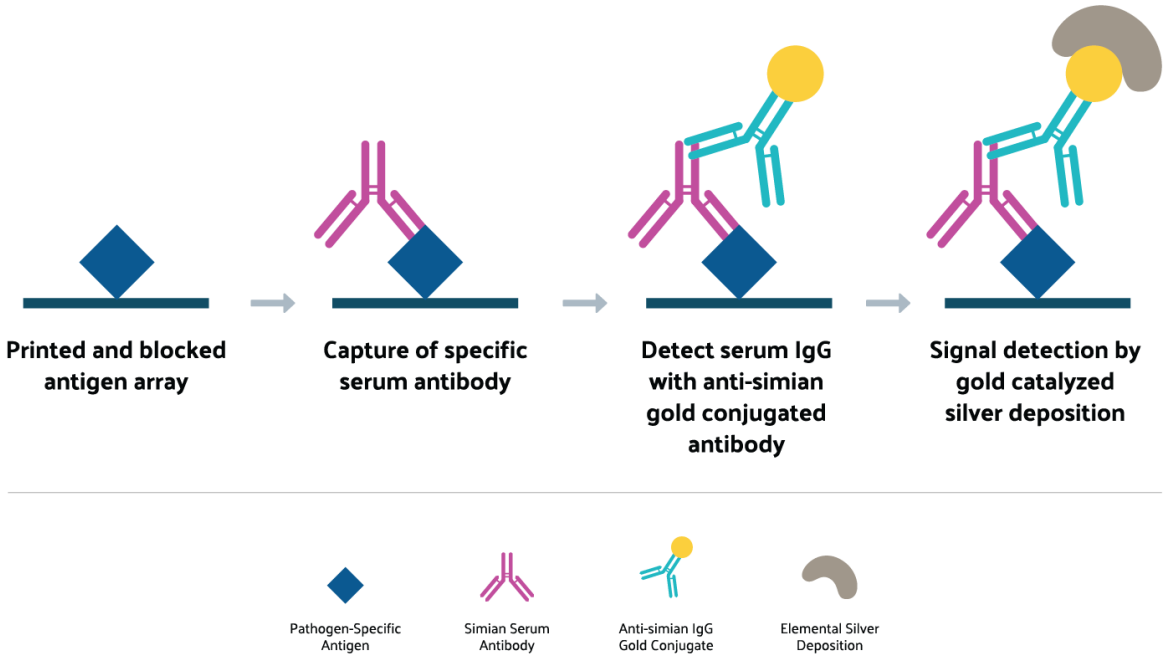
1.0 Introduction

1.1 CSA: Simian Overview

The health and well-being of non-human primate (NHP) colonies are critical to research success. The Colony Surveillance Assay™: Simian kits provide a sensitive, robust, and user-friendly alternative to ELISA or bead-based methods by combining multiplex detection technologies with compact and cost-effective analysis tools for colony health screening. This approach provides an indication of previous exposure to pathogenic agents present in NHP colonies, both to identify potential infectious threats to the colony and their human caretakers and to ensure research remains uncompromised.

The CSA: Training Kit is intended to provide laboratory personnel with a learning experience prior to running real samples on a CSA kit. Critical steps, like the SilverQuant reagent addition, are highlighted to emphasize the importance of practice. Each CSA: Training Kit includes reagents to run one full 96-well plate as a practice run. Developed plates are scanned on the IAN System and analyzed with the 12-9000 CSA Training analysis file.

Figure 1. CSA: Simian Schematic



1.2 SilverQuant Surface Chemistry

The CSA: Training Kit arrays are printed on Intuitive Biosciences' proprietary protein-binding plates specifically designed for multiplex immunoassays. These arrays deliver a high signal-to-noise ratio with high sensitivity for protein microarray applications.

2.0 Kit Contents

Component	Description	Quantity	Prod. No.
CSA: Simian Training Plate	Plate containing 96 wells of Reagent Controls	1 plate	12-9001
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
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
CSA: Training Kit User Protocol			L804

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
15 mL conical tubes	Various
50 mL conical tubes	Various
Reagent troughs	Disposable reagent troughs capable of holding at least 50 mL
Deionized or Ultrapure Water	Clean water
Microarray Scanner	IAN System
Microarray Image Analysis Software	QuantoPic software (included with IAN System)
CSA: Training Kit data analysis template	Included with QuantoPic software
Micropipettes	Single, Repeat, and 8-channel; various capacities

4.0 Storage

The CSA: Training Kit (Prod. No. 12-9000) 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. Intuitive Biosciences recommends the use of gloves, lab coats, and eye protection when working with any material.

6.0 Protocol Overview

The CSA: Training Kit contains sufficient reagents for a full 96 well plate. However, only a single assay may be performed with the reagents included in the kit. Reagent B is air sensitive and once opened and must be used within 24 hours Both Reagent A and Reagent B are light sensitive and should not be exposed to direct or excess light.

CSA: Training Plates should be handled with care. Never touch the bottom of the well and do not allow plates to dry once they have been wetted. 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 Addition of CSA Buffer to the Array

The CSA: Training Kit does not contain serum samples. When running a full CSA: Simian Panel A kit, this step would include the preparation of a dilution plate and the addition of 5 µL of serum for each sample into each well of the dilution plate. Because there are no samples included in the CSA: Training Kit the incubation time is decreased from **1 hour to 10 minutes.**

NOTE: After step 7.2.2, do not allow the surface of the array to dry completely at any time before scanning the plate.

7.2.1 Pipette 150 µL of CSA Buffer (Product No. 7-1037) into all wells of the CSA: Simian Training Plate (Product No. 12-9001).

7.2.2 Cover the wells with a plate seal or box lid to prevent evaporation. Tap the side of the plate and incubate at room temperature for **10 minutes**.

7.3 Prepare Gold Conjugate

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 tap to mix.

7.3.2 Prepare the Gold Conjugate Reagent by adding 90 µL of SilverQuant Anti-Simian IgG Gold Conjugate to 18 mL of CSA Buffer (Product No. 7-1037). Mix gently and thoroughly.

7.3.3 Prepare a fresh reagent trough for Gold Conjugate and pour diluted Gold Conjugate into trough.

7.3.4 Pour fresh 1X Slide Wash Buffer (SWB, prepared in **Step 7.1.1**) into fresh reagent trough.

7.4 Remove Buffer (faux sample) from Well

7.4.1 Remove CSA Buffer (or "Sample") from plate by inverting over a liquid waste container.

7.4.2 Invert plate and tap onto pile of paper towel to remove any residual buffer.

7.5 Perform Wash 1 and Add Gold Conjugate Reagent

7.5.1 Add 150 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 waste container.

7.5.2 Repeat step 7.5.1 two more times, adding 150 µL of 1X Slide Wash Buffer for a total of 3 washes in the plate.

7.5.3 Remove the final wash and immediately add 150 µL Gold Conjugate Reagent (prepared in **Step 7.3.2**). Tap side of the plate to ensure the bottom of the wells are completely covered.

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

7.6 Perform Wash 2 and Develop Plate

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

7.6.2 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.

7.6.3 Repeat **Step 7.6.2** 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. Perform the following steps out of direct sunlight.

It CRITICAL to add the SilverQuant Reagent A and B mix quickly to the CSA: Simian Training Plate because the reaction is time dependent. Once Reagent A and B have been mixed together and added to the reagent trough add them quickly to the plate using a multi-channel or repeat pipettor.

Read and understand **Steps 7.6.4 -7.6.9** and prepare all equipment needed before beginning.

7.6.4 Prepare by setting a timer to 3 minutes, but do not press Start.

7.6.5 Add pipet tips to a multichannel or repeat pipettor and prepare it to dispense 100 µL. Obtain a fresh reagent trough capable of holding 20 mL of liquid.

7.6.6 Prepare the SilverQuant Development Reagent by directly pouring SilverQuant Reagent A (Product No. 10-2132) into the SilverQuant Reagent B bottle (Product No. 10-2112). Replace the rubber stopper in the glass bottle and shake vigorously for approximately 3 seconds and pour into reagent trough.

7.6.7 Quickly remove Slide Wash Buffer from the wells by inverting plate over liquid waste. Tap plate upside down on a pile of paper towel to remove excess liquid. **Immediately** add 100 µL of the SilverQuant Development Reagent to each well of the CSA: Simian Training Plate using the multichannel or repeat pipettor. Tap the side of the plate twice to ensure that the Development Reagent covers the entire bottom of each well of the plate.

7.6.8 Immediately start the timer and incubate for **exactly 3 minutes**. Place an opaque cover (i.e. the lid of a box) over the CSA: Simian Training Plate to protect it from light.

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

7.7 Final Rinse

7.7.1 After 3 minutes have elapsed, remove the Development Reagent from the CSA: Simian Training Plate by inverting over a proper chemical waste container. Immediately fill the wells with ultrapure water using the squirt bottle. Remove water by inverting plate over sink or liquid waste.

7.7.2 Repeat the water flush twice to ensure all the Development Reagent is rinsed out of the plate.

7.7.3 Dry the CSA: Simian Training Plate with plate centrifuge by inverting the plate over a paper towel and gently spinning down the plate. Alternately, the plate may air dry on the benchtop.

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

7.8 Data Collection and Analysis

7.8.1 Once the CSA: Simian Training Plate is completely dry, insert it into the IAN System and open the QuantoPic software.

7.8.2 Select the 12-9000 CSA Training option from the experiment menu. Since all training plate wells are identical, press the Set Manually button, Check All, and select OK.

7.8.3 Press Start to initiate plate scanning and software analysis. The IAN System will automatically output the scan if no errors were found. If spot imaging errors occur, refer to section the IAN System User Manual.

7.8.4 A folder containing the Excel output, a PDF report, and scanned well images is generated at the conclusion of a successful scan.

8.0 Ordering Information

Telephone: +1.608.561.8730

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

Website: www.intuitivebio.com

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This product uses SilverQuant® technology available exclusively from Intuitive Biosciences, Inc. This product and/or the use of this product may be covered by one or more patents of EPPENDORF AG, including, but not restricted to, the following: US Patent #7,321,829 and European Patent #1179180B1.
Intuitive Biosciences' thin nitrocellulose protein microarray slide 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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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 omitted or a reagent was mishandled – review process and reagents described in the protocol.
2. A low signal is seen in the positive controls - anti-simian IgG gold conjugate solution was missing or mishandled.

Problem: Heterogeneous Background

Suggested Causes & Solutions:

1. A washing/drying artifact is seen when salts in the wash buffer leave “streaks” in the array image - briefly rinse wells with purified water and dry immediately.
2. Dust has adhered to the well after it has been dried – blow or wipe off any dust that may have settled into a well.