Table of Contents

1.0 Introduction

SilverQuant Detection Kit Overview

SilverQuant detection technology, shown schematically in Figure 1, is an array-based method for the detection of biotin labeled proteins with exceptional sensitivity, reproducibility and dynamic range. SilverQuant (SQ) reagents generate light grey-to-black spots that are visible to the naked eye and are easily detectable using affordable chromogenic scanners. SilverQuant detection is based on gold particle-enhanced silver deposition and is several times more sensitive than fluorescence. It is not subject to photobleaching, ozone sensitivity, or spot blooming. A single sample well can contain as many as several hundred highly localized array spots that can be archived for years after testing. SilverQuant detection technology works with AQ 1000 scanning systems, a complete platform that integrates chromogenic scanning and array analysis at a price that is comparable to that of a bench-top ELISA system. AQ System supports a wide variety of assay types including quantitative antibody sandwich assays (multiplex ELISAs), profiling sandwich assays, high-density-based antibody arrays, as well as arrays comprised of peptides, proteins, and tumor lysates.

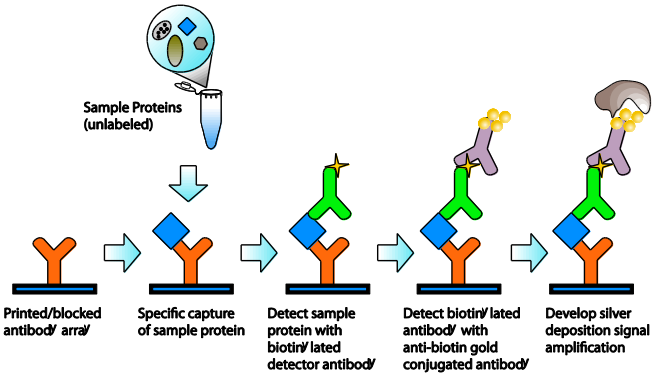


Figure 1. Schematic showing the configuration of a multiplexed sandwich assay using SilverQuant detection

2.0 Kit Contents

Component	Description	Quantity	Product No.
SQ Anti-biotin Conjugate	Antibody to biotin labeled with gold nanoparticles.	40 µL	10-2128
SQ Reagent A	Solution containing silver salt used in generating the chromogenic signal.	10 mL	10-2132
SQ Reagent B	Solution containing reductant used in generating the chromogenic signal.	10 mL	10-2112
SQ Unibuffer	Buffer concentrate used in the chromogenic assay.	15 mL	10-2125
SQ Buffer	Buffer concentrate used in the chromogenic assay.	5 mL	10-2126
SQ Reagent C	Minimizes non-specific binding to the microarray.	200 mg	10-2135
Tween® 20	Surfactant used in preparation of the Wash buffer.	1 mL	10-2116
SQ Array Tube	Device used to incubate 1-4 microarray slides with chromogenic reagents.	1 Tube	10-2117
SilverQuant Detection Kit-Clear Surface User Protocol		L086	

3.0 Materials Required, But Not Included

Component	Description
Orbital plate shaker	Capable of 160 RPM.
Bench-top microcentrifuge	Capacity to hold 1.5 mL microcentrifuge tubes.
Microcentrifuge	Capable of spinning 3 x 1" microscope slides.
Nitrogen, or Purified Air Stream	With regulator set at 80 psi for drying slides.
Vortex mixer	For mixing samples.
1.5 mL Microcentrifuge tubes	Used for diluting serum samples.
200-500 mL Container	Used for holding wash buffer.
Squirt bottle	Fill bottle with ultrapure water for final rinse step
Ultrapure water	Resistivity >18.0 MΩ·cm at 25°C
Micropipettes	Single, Repeat, and 8-channel; various capacities

4.0 Storage and Stability

The SilverQuant Detection Kit should be stored at 2-8°C until used.

5.0 Safety and Handling

Use Universal Safety Precautions whenever handling any human body fluids. For all other materials, normal precautions exercised in handling laboratory materials should be followed. The chemical, physical, and toxicological properties of this product may not have been thoroughly investigated. We recommend using gloves, lab coats, and eye protection when working with any material.

The following risk and safety phrases apply to components of the kit:

SQ Reagent A	SQ Reagent B
R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. **S61** Avoid release to the environment. Refer to special instructions/safety data sheets.	Harmful **R40** Limited evidence of a carcinogenic effect. **R43** May cause sensitization by skin contact. **R68** Possible risks of irreversible effects. **S26** In the event of eye contact, rinse immediately with plenty of water and seek medical advice. **S36/37/39** Wear suitable protective clothing, gloves and eye/face protection.

For more information, please consult the appropriate material safety data sheets, available from Intuitive Biosciences. Please note Reagent B is stored in the presence of nitrogen and is oxygen sensitive. Only use Reagent B the same day as when the container is opened and do not reuse any which may remain after the completion of the assay.

6.0 Assay Considerations

6.1 The silver precipitation step (incubation with the mix of SQ Reagent A and B) is the most crucial step of the protocol. Please follow the protocol carefully to ensure consistent results.

6.2 The SQ detection kit should be performed at room temperature (18-30°C). Silver precipitation performed after mixing SQ Reagent A and B (Detection Solution) is sensitive to temperature.

- 6.2.1 At temperatures higher than 30°C, the rate of reaction will increase with higher background.
- 6.2.2 At temperatures lower than 18°C, the rate of the reaction will decrease.
- 6.2.3 If your lab is at 18-30°C, all reagents should be allowed to equilibrate to room temperature before using.
- 6.2.4 If room temperature is outside the recommended range, the SQ Reagent A and B reagents should be placed in a waterbath at 22°C and allowed to equilibrate before using.

6.3 After mixing SQ Reagent A and B, the solution should be protected from light to help avoid high background. The SQ Array Tube protects the solution from light. Alternately, if you are performing the precipitating reaction in the wells of a microtiter plate (Appendix A), a black microplate lid, or similar cover that shields the arrays from light, should be used.

6.4 The presence of dust and metallic residues on slides should be avoided because they can lead to an increased background. Use clean glass or plastic disposable pipettes.

6.5 During the processing of slides, do not allow the arrays to dry at any point.

6.6 Use care when handling the slides. Scratches in the slide can lead to aggregation of silver precipitate and interfere with results. Allowing the substrate to slide across a surface (such as a benchtop) may cause scratching on the bottom and should be avoided.

7.0 SilverQuant Detection Kit Procedure

7.1 General Information and Workspace Preparation

7.1.1 General Information

The chromogenic assay begins after a biotin-labeled detector molecule (such as a biotinylated antigen-specific antibody or biotinylated anti-IgE) has been captured for detection of the analyte(s) of interest. For example, because of its biotin-binding properties, SilverQuant detection can replace detection using labeled streptavidin. If you are performing the assay for the first time, we recommend using the SQ Array Tube for the Detection Step.

If you are using a sample separator such as SIM*plex*™ and you plan to use the SQ Array Tube for the Detection Step (Step 7.3.2), you will need to remove the slides from the sample separator. Do not allow the arrays to dry during removal or at any point during the processing of slides. Be careful not to touch or scratch the arrays when transferring the slides to the SQ Array Tube.

A method is now available to enable performing the complete SQ detection procedure in the wells of a 16-well separator or modified 96-well device without using the SQ Array Tube (see Appendix A). Use of this method is required if you are using single-use 16-well adhesive gaskets. Do not allow the arrays to dry at any point during processing of slides. If you are using 16-well adhesive gaskets, slides can be assembled in a 4-slide holder (not included with the reagent kit) during the assay for easier handling.

7.1.2 Workspace Preparation

7.1.2.1 We recommend placing a plastic or paper protective coating on the benchtop as SQ Reagent A is a strong staining agent.

7.2 SilverQuant Detection Reagent Preparation

7.2.1 Wash Buffer

7.2.1.1 To prepare 200 mL of Wash Buffer, dilute 5 mL of UniBuffer with 195 mL of ultrapure water. Add 200 µL of Tween 20 and mix well.

7.2.2 Pre-Blocking Buffer

7.2.2.1 To prepare 20 mL of Pre-blocking Buffer, dilute 5 mL of UniBuffer with 15 mL of ultrapure water. Open the Reagent C tube (blue cap) and dissolve the contents in the 20 mL of diluted UniBuffer.

7.2.3 Conjugate Buffer

7.2.3.1 To prepare 20 mL of Conjugate Buffer, dilute 2 mL of Pre-blocking Buffer (prepared as in step 7.2.2.1) in 18 mL of ultrapure water.

7.2.4 Rinse Buffer

7.2.4.1 To prepare 40 mL of Rinse Buffer, dilute 4 mL of SQ Buffer in 36 mL of ultrapure water.

7.2.5 SQ Reagent A and SQ Reagent B

7.2.5.1 Before starting the experiment, these solutions have to be removed from the refrigerator and warmed to room temperature (18-30°C). If room temperature is outside of this range, place both reagents in a water bath at 22°C for optimal results. The closer these reagents are to this temperature when they are mixed and used, the better.

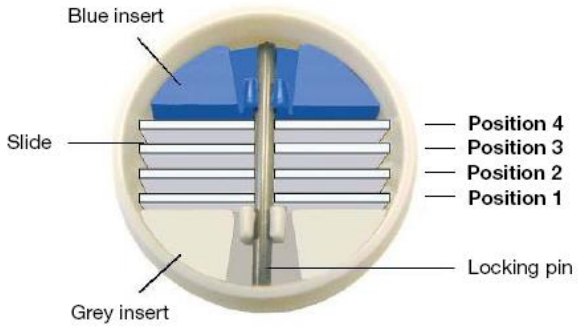
7.3 Incubation Using SQ Detection Reagents and the SQ Array Tube

7.3.1 Processing Slides After Application of Biotinylated Detection Reagents

7.3.1.1 Obtain 1-4 slides processed up to the application of biotinylated detection reagents. Before starting this step, a biotin-labeled detector molecule (such as a biotinylated antigen-specific antibody or biotinylated secondary antibody) must be captured to enable detection using SQ Chromogenic reagents. Slides must be removed from the sample separator, but do not allow them to dry.

7.3.2 Positioning and Insertion of Slides in the SQ Array Tube

7.3.2.1 Open an SQ Array Tube and remove the locking pin from the tube with forceps. Position slides in the SQ Array Tube as described in Step 7.3.2.2-7.3.2.5 to avoid the arrays facing an adjacent surface too closely.



7.3.2.2 **If 1 slide is used:**

- Insert it in position 3, with arrays facing the blue insert.
- The volume of liquid to be dispensed into the tube for all steps is 17 mL.

7.3.2.3 **If 2 slides are used:**

- Insert the first slide into position 4, with arrays facing the grey insert.
- Insert the second slide into position 3, with arrays facing the blue insert.
- The volume of liquid to be dispensed into the tube for all the steps is 15 mL.

7.3.2.4 **If 3 slides are used:**

- Insert the first slide into position 4, with arrays facing the grey insert.
- Insert the second slide into position 3, with arrays facing the blue insert.
- Insert the third slide in position 2, with arrays facing the blue insert.
- The volume of liquid to be dispensed into the tube for all the steps is 13 mL.

7.3.2.5 **If 4 slides are used:**

- Insert the first slide into position 4, with arrays facing the grey insert.
- Insert the second slide into position 3, with arrays facing the blue insert.
- Insert the third slide in position 2, with arrays facing the blue insert.
- Insert the fourth slide in position 1, with arrays facing the blue insert.
- The volume of liquid to be dispensed into the tube for all the steps is 11 mL.

7.3.2.6 Lock the slides in the tube with the black locking pin. The slides will stay in the SQ Array Tube until the drying step (Step 7.3.4.4 of this protocol). Use a 50 mL conical tube

holder to stabilize the tube during steps where you need to keep it in a vertical position, if desired.

7.3.3 Incubation With Anti-biotin Gold Conjugate

7.3.3.1 Wash the slides 3 times by:

- adding the appropriate amount of Wash Buffer to the SQ Array Tube,
- waiting 1 min (invert tube once at beginning and end of incubation), and
- emptying the tube into a sink.

7.3.3.2 Incubate the slides in Pre-blocking Buffer for 10 min at room temperature. Invert tube at beginning and end of incubation. During incubation, keep the tube in a vertical position.

7.3.3.3 During the pre-blocking incubation period, prepare a 1:500 dilution of the anti-biotin Gold Conjugate in Conjugate buffer.

- For example, add 40 µL of anti-biotin Gold Conjugate to 19.96 mL of Conjugate buffer.

NOTE: since condensate often collects on the cap of the anti-biotin Gold Conjugate tube, briefly centrifuging the tube is recommended to insure that the conjugate is at the proper concentration before using. You may also want to optimize the dilution of the gold conjugate for your assay, as 1:500 is simply a good starting place for most people.

7.3.3.4 After emptying the tube of Pre-blocking Buffer, incubate the slides in the 1:500 anti-biotin Gold Conjugate solution for 45 min at room temperature (18-30°C) while mixing at 300 rpm. The SQ Array Tube must be closed firmly and placed in a horizontal position on the Thermomixer such that the "This side up" sticker is facing up. If a Thermomixer (or other shaker) is not used, leave the SQ Array Tube *vertical* and mix the tube every 15 min by inverting twice.

7.3.3.5 Empty the tube after incubation with the anti-biotin gold conjugate. Place the tube vertically and wash the slides four times for 1 min in Wash Buffer, inverting the tube twice during each wash step to rinse the cap of any remaining conjugate.

7.3.4 Incubation With SQ Reagent A & B

7.3.4.1 Incubate the slides in Rinse Buffer for 1 min in the SQ Array Tube with the screw cap closed. Invert the tube twice and empty the SQ Array Tube completely. Shake the tube to remove as much Rinse buffer as possible.

7.3.4.2 Repeat Step 7.3.4.1.

7.3.4.3 Confirm that SQ Reagent A & B have been allowed to equilibrate to the proper temperature (18-30°C). Fill the tube with the appropriate amount of SQ Reagent B (see below). Add an equal volume of SQ Reagent A and firmly close the SQ Array Tube. Incubate the slides with the Reagent A + B mixture for exactly 5 min, shaking vigorously for the first ten seconds to mix the two reagents.

- **If 1 slide is used,** use 8.5 ml of Reagent B + 8.5 mL Reagent A.
- **If 2 slides are used,** use 7.5 ml of Reagent B + 7.5 mL Reagent A.
- **If 3 slides are used,** use 6.5 ml of Reagent B + 6.5 mL Reagent A.
- **If 4 slides are used,** use 5.5 ml of Reagent B + 5.5 mL Reagent A.

7.3.4.4 Agitate the firmly closed tube horizontally at 300 rpm at room temperature (18-30°C) during the 5 min incubation with the staining solution. The sticker on the SQ Array Tube should be facing up. If a shaker is not used, keep the tube in the *horizontal* position at room temperature with the sticker facing up. Mix the staining solution by vigorously shaking the tube once each minute, and then return tube to the horizontal position.

7.3.4.5 Discard the staining solution (solution should be gray or pale purple in color) into an appropriate waste container and wash slides twice for 1 min with ultrapure water at room temperature, then disengage the locking pin and remove slides from the SQ Array Tube.

NOTE: the staining solution is hazardous to aquatic organisms and should not be released into the environment. Consult the appropriate MSDS and your local regulations for more information on proper disposal of silver waste.

7.3.4.6 Wipe the back side of the slides (covered with a thin coating of non-specific silver precipitate) using a paper towel, then immediately centrifuge the slides in an appropriate holder (*e.g.* Eppendorf CombiSlide adapter) for 5 min at 600 rpm to dry them. Alternatively, the slides may be dried using compressed gas. Take care not to touch the arrays or scratch the slide(s).

8.0 Ordering Information

Telephone:	608.204.8730
Toll Free in North America:	888.700.7442
Fax:	608.204.8740
E-mail:	orders@intuitivebio.com
Website:	www.IntuitiveBio.com

Products to any third party. Purchaser agrees that Purchaser shall not have the right to authorize any third party to use or sell any Products or derivatives thereof. Unless a license has been executed between Purchaser and Intuitive Biosciences® Biosciences, Inc. explicitly providing otherwise, Purchaser will not: (i) reformulate or create derivatives of the Products; or (ii) use the Products for providing services to a third party (e.g., screening or profiling); (iii) use the Products in a diagnostic process; (iv) use the Products in a quality control or quality assurance process for the manufacture of a product for sale; or (v) use the products as a component of a kit.

9.0 Appendix A: Detection Using SilverQuant Detection Reagents in Wells

NOTE: the procedure below has not been completely optimized, so assay performance may not be equivalent to assays performed using the SQ Array Tube.

Additional Materials Required for Using SilverQuant Chromogenic Reagents in the Wells

Component	Description/Recommended Source
Multi-channel repeat pipettor	8 or 12-channel repeat pipettor
Multi-channel pipettor reagent boat	NA
50 mL Conical tube	NA

9.1 Run your assay up to the point where the wells are washed following incubation with biotinylated detector reagent.

9.2 Wash by adding 150-200 µL of Wash Buffer to each well with a multi-channel pipettor. No agitation or incubation time is needed. Remove liquid from the sample separator by inverting over a sink and then shaking.

9.3 Repeat wash step at least two additional times. After washing proceed immediately to next step. Do not allow the surface to dry.

9.4 Add 150 µL of Pre-blocking Buffer to each well. Incubate the slides for 10 min at room temperature. No agitation is needed. During this incubation step, prepare the required volume of diluted anti-biotin Gold Conjugate. The anti-biotin Gold Conjugate must be diluted 1:500 in the Conjugate Buffer (0.1 mL per well is needed, plus some extra that will remain in the reagent boat).

9.5 Remove Pre-blocking Buffer from the sample separator by inverting over a sink and then shaking vigorously. Proceed immediately to next step. Do not allow the surface to dry.

9.6 Add 70-100 µL of prepared anti-biotin Gold Conjugate to each well with a multi-channel pipettor. Cover and incubate the slides for 45 min at room temperature while shaking.

9.7 After incubation with the anti-biotin Gold Conjugate is complete, remove liquid from the sample separator by inverting over a sink and then shaking. Proceed immediately to the next step. Do not allow the surface to dry.

9.8 Wash by adding 150-200 µL of Wash Buffer to each well with a multi-channel pipettor. No agitation or incubation time is needed. Remove liquid from the sample separator by inverting over a sink and then shaking vigorously. Proceed immediately to the next step.

9.9 Repeat the previous step at least three additional times. Afterward, proceed immediately to next step. Do not allow the surface to dry.

9.10 Rinse the arrays twice by adding enough Rinse Buffer (~300 µL) to each well that the buffer overflows from the wells. Remove Rinse buffer by inverting the device over a sink. Wipe off any remaining Rinse buffer from the top of the sample separator with a paper towel.

9.11 Confirm that SQ Reagents A & B have reached room temperature (18-30°C). Add 50 µL of Reagent B to each well using a multi-channel pipettor (a multi-channel *repeat* pipettor works best because it allows you to add the reagent more quickly).

9.12 Using a multi-channel repeat pipettor, add 50 µL of Reagent A to each well as quickly as possible. Make sure to complete addition of Reagent A to all wells within 20 seconds, as it is important that the staining time be as close to identical as possible for all wells. Immediately cover the sample separator with black microplate lid (or similar barrier to light) and incubate for 5-10 minutes with rocking at room temperature (18-30°C).

NOTE: it is recommended that you start with a 5 minute incubation of the staining solution with the slide. Increasing the incubation time can lead to stronger signal from your array spots, but the background may also increase, so longer incubation times may not yield better results.

9.13 After incubation with SQ Reagent A & B, remove liquid from the sample separator by inverting and then gently shaking over a waste container. Proceed immediately to the next step. Do not allow the surface to completely dry.

NOTE: the staining solution is hazardous to aquatic organisms and should not be released into the environment. Consult the appropriate MSDS and your local regulations for more information on proper disposal.

9.14 Add 150-200 µL of ultrapure water to each well. No agitation is needed. Remove liquid from the sample separator by gently shaking the device over a sink.

9.15 Repeat previous step.

9.16 Dry the slide by either centrifuging or blowing compressed gas into the wells.

9.17 Scan and analyze the slide.