Q-Plex™ Mouse Cytokine Array
Stripwell™

FOR RESEARCH USE ONLY
Catalog Number: 599400

Version 3.4
Quansys Biosciences is dedicated to the development of protein arrays to aid researchers and clinicians in better understanding, diagnosing, and treating disease for the betterment of people’s lives. Previous tests that have been developed include arrays for the quantitation of cancer markers, auto-reactive antibodies, Pneumococcal serotype-specific antibodies, cardiac markers, pituitary hormones, and human cytokines.
Important Precautions

Read all of the Instructions before beginning test.

1) Before beginning the assay, the user must insure that the CCD imager has been set-up in accordance with the instructions on page 8 of this manual. Only after setting up the imaging parameters, should the user begin the assay.

2) Do not mix any components of this kit with the components from other kits, unless the instructions state otherwise.

3) Storage: Always keep the unopened kit containing the 96-well plate, Antigen Standard, Detection Mix, Streptavidin HRP, Substrate A, Substrate B, Sample Dilution Buffer, and Wash Buffer refrigerated at 4°C while not in use. After opening the kit and reconstituting the reagents, observe the stability recommendations stated on page 9 (Kit Component Reconstitution).

4) Prevent contamination by following instructions exactly, using properly functioning equipment and practicing aseptic techniques.

5) Do not expose the kit components to heat greater than 26°C.

6) Perform the test at room temperature: 20° - 25°C.

7) Use new pipette tips whenever dispensing a new component.

8) Keep plate sealed until use. If using plates for multiple tests, keep unused stripwells sealed with plate covers supplied in kit. Place the stripwells back into the Mylar bag provided to prevent the stripwells from drying out.
Introduction

The immune system is a dynamic symphony of many cell types employed in host protection, regeneration, and regulation. This system is intended to protect the host from foreign and native factors. To work in concert, the immune system must communicate with exactness. Cytokines, hormone-like proteins produced by immune cells, act as the messengers of communication. Through cytokines, the immune system regulates stem cell differentiation, inflammation, the type of immune response executed, angiogenesis, and many other events. Because of these various activities, cytokines are also involved in progression and pathogenesis of most diseases.

Most cytokines perform multiple functions and often share the same receptors. A single cytokine can elicit one effect on one cell type, and a completely different effect on another cell type, then induce a completely different response when administered concomitantly with other cytokines. This diversity of activity is what allows the immune system to control a great variety of events with a relatively small repertoire of signaling agents. As a result, the profile of type and concentration of cytokines present at a specific time is critical to receiving the desired function. Because these profiles define what is happening in the immune system, and because of cytokine involvement in both disease pathogenesis and disease recovery, the monitoring of their levels is an invaluable tool in understanding, diagnosing, and monitoring disease.

Cytokines are conventionally detected using different types of reporter-linked immunoassays. The most accepted way is by sandwich (capture) ELISA. This process uses two antibodies. One antibody is first bound to a solid phase (i.e. micro-well plate). The sample is incubated on the surface, allowing the cytokine and bound antibody to associate. Following this, each well is incubated with a second enzyme-linked antibody. The second antibody reacts with a different region of the cytokine. This reaction is detected by monitoring the activity of the enzyme, which is proportional to the amount of cytokine present in the sample.

Conventional ELISAs, though allowing the researcher to test many samples at one time, can test only one type of cytokine in a single test. This makes testing multiple cytokines costly with respect to time, reagents, and sample volume. These factors and the importance of profiles when working with cytokines have prompted many researchers to develop cytokine tests in multiplexed formats. These formats have included fluorescent bead-based assays and slide-based protein arrays. These methodologies, in contrast to the conventional ELISA, allow the researcher to test many cytokines at one time, but test only one or a few samples at a time, reducing the throughput of the assay.

The Q-Plex™ Mouse Cytokine Stripwell™ Array is a fully quantitative ELISA-based test where 16 distinct capture antibodies have been absorbed to each well of a 96-well plate in a defined array. Using less than 30 µl of sample, up to 84 different samples can be assayed for all 16 cytokines in less than 2.5 hours. Sensitivity is system dependent. It typically ranges between 30 pg/ml to less than 1 pg/ml. All of the antibodies used in this array have been subject to a rigorous and comprehensive cross reactivity protocol and verified to be non-cross reactive with any other system on the array.

The Q-Plex™ Mouse Cytokine Stripwell™ Array is an invaluable tool for the simultaneous detection of multiple cytokines, providing a platform that overcomes the shortfalls of both conventional assays and predecessor multiplex assays to provide the researcher an easy and cost effective means of exploring the complexity and range of cytokine responses.
Graphical Representation

Array Platform

Each well has 16 spots. The diagram below depicts the cytokine location in each well of the 96-well plate.
Array Performance

Sensitivity:
The Lower Limits of Detection (LLD) were determined for each system. The LLD is the mean of the background plus 2 standard deviations of the background.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Range (pg/ml)*</th>
<th>LLD (pg/ml)**</th>
<th>Standard (pg/ml)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>125-7.8</td>
<td>≤2.5</td>
<td>125</td>
</tr>
<tr>
<td>IL-1β</td>
<td>490-30.6</td>
<td>≤5.5</td>
<td>490</td>
</tr>
<tr>
<td>IL-2</td>
<td>90-5.6</td>
<td>≤2.0</td>
<td>90</td>
</tr>
<tr>
<td>IL-3</td>
<td>110-6.9</td>
<td>≤1.0</td>
<td>110</td>
</tr>
<tr>
<td>IL-4</td>
<td>115-7.2</td>
<td>≤1.0</td>
<td>115</td>
</tr>
<tr>
<td>IL-5</td>
<td>50-3.1</td>
<td>≤2.5</td>
<td>50</td>
</tr>
<tr>
<td>IL-6</td>
<td>140-8.8</td>
<td>≤2.0</td>
<td>140</td>
</tr>
<tr>
<td>IL-9</td>
<td>2600-162.5</td>
<td>≤36.5</td>
<td>2600</td>
</tr>
<tr>
<td>IL-10</td>
<td>480-30</td>
<td>≤5.5</td>
<td>480</td>
</tr>
<tr>
<td>IL-12</td>
<td>11-0.7</td>
<td>≤1.5</td>
<td>11</td>
</tr>
<tr>
<td>MCP-1</td>
<td>500-31.3</td>
<td>≤24.0</td>
<td>500</td>
</tr>
<tr>
<td>IFNγ</td>
<td>1000-62.5</td>
<td>≤20.0</td>
<td>1000</td>
</tr>
<tr>
<td>TNFα</td>
<td>430-26.9</td>
<td>≤5.0</td>
<td>430</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>1980-123.8</td>
<td>≤10.0</td>
<td>1980</td>
</tr>
<tr>
<td>GMCSF</td>
<td>90-5.6</td>
<td>≤1.5</td>
<td>90</td>
</tr>
<tr>
<td>RANTES</td>
<td>120-7.5</td>
<td>≤2.5</td>
<td>120</td>
</tr>
</tbody>
</table>

*If standard curves that approach the LLD for the cytokines are desired, it is recommended that the user continue 2-fold dilutions of the Antigen Standard.

**Please note that these LLD values are minimum QC requirements. The LLDs for any released lot will be as good as or better than these values.

***This is the concentration of the Antigen Standard when reconstituted with 300 μl. This will be the concentration of the high point of the standard curve.

For assay performance statistics, please review the Certificate of Analysis that accompanied your kit.
Kit Contents

1) 96-Well Stripwell Microplate (12 8-well strips): Pre-spotted and pre-blocked 96-well stripwell microplate with 16 different cytokine capture antibodies in each well.
   a. To view the quality assurance image, visit our website at www.quansysbio.com, and be prepared to enter your plate code.
2) Antigen Standards (3)
   a. When comparing results from this kit to other platforms, it is recommended that the user test the same antigen standards and samples on both platforms. This is to validate the accuracy of the standard from one platform to another.
3) Detection Mix
4) Streptavidin-HRP Concentrate
5) Streptavidin-HRP Dilution Buffer
6) Substrate - for chemiluminescence. Do not mix until instructed.
   a. Substrate A
   b. Substrate B
7) Sample Dilution Buffer
   a. See Appendix B for information regarding compatible buffers and array inhibitors.
8) Wash Buffer 20X
9) Plate Seals (2)
10) Mouse Pad (First time buyer only.)

Required Supplies

*These items are required, but not included in the kit.*

1) 8 or 12 channel pipette (20-200 µl) and/or one channel pipette and tips
2) Automatic stripwell microplate washer, or Multi-channel pipette
3) CCD based Imager (or equivalent)
4) Orbital shaker
Video Manual

1) A video demonstration on each step of the assay is available to download at www.quansysbio.com.
2) If a high-speed internet connection is not available, please contact us for a free copy.
   Toll-free 1-888-782-6797.

Setup of CCD Imager

This must be optimized before beginning the test.

1) Set the f-stop at the lowest setting on the camera. (The aperture is fully opened.)
2) Open the imaging software and set to expose preview.
3) Using the provided mouse pad as a guide, open the imager box and place the mouse pad inside the field of view. Turn on the white light in the box, or keep the box door open to allow light in while adjusting the focus. With the aperture fully opened, the mouse pad should be visible in the imaging software.
4) Adjust the zoom on the camera so that the image of the plate takes up 80% of the field of view in the imaging software.
5) Adjust the focus on the camera so that the individual spots on the plate are visible and clear in the imaging software.
6) Check the settings on the camera to make sure that it is not set to bin pixels.
7) Make sure the imager box is completely light sealed, as the response is chemiluminescent.

Note: When imaging the array, small adjustments may need to be made to the camera focus.

For more details on specific imaging systems, please visit our website at www.quansysbio.com or contact Technical Support at 1-888-782-6797.

Setup of Plate Washer

1) Determine the method of washing you are going to use.
   a. Automatic stripwell microplate washer (see Appendix A)
   b. Multi-channel pipette (see Appendix A)
2) Each method will effectively wash the plate. Choose the method most convenient for your lab.
Kit Component Reconstitution & Stability

Note: If sterile deionized water is not available, then ultra pure or deionized water is acceptable.

Store unopened kit at 4°C until ready to use.
Follow the suggested storage guidelines after opening the kit and reconstituting the reagents.

1) Antigen Standard
This will be used either as a control or to construct a standard curve.
   a. To the vial labeled Antigen Standard add 300 µl sterile deionized water.
   b. Vortex the vial until the lyophilized Antigen Standard is fully reconstituted.
   c. Store unused Antigen Standard at -20°C. Good for 1 week.

2) Sample Dilution Buffer
   a. To the vial labeled Sample Dilution Buffer add 10 ml of sterile deionized water.
   b. Vortex the vial until the lyophilized Sample Dilution Buffer is fully reconstituted.
   c. Mix solution thoroughly by hand for 30 seconds to make sure that the buffer is a homogeneous mix.
   d. Mouse samples must be diluted prior to use to prevent interference by mouse serum.
   e. Store unused Sample Dilution Buffer at 4°C. Good for 6 months.

3) Detection Antibody Mix
   a. To the vial labeled Detection Mix add 3.5 ml of sterile deionized water.
   b. Vortex the vial until the lyophilized Detection Mix is fully reconstituted.
   c. Store unused Detection Antibody Mix at 4°C. Good for 1 week.

4) Streptavidin-HRP
   a. Quickly spin down the Streptavidin-HRP Concentrate to make sure that the solution is not on the sides of the vial.
   b. Add 2 µl of Streptavidin-HRP Concentrate to the vial labeled Streptavidin-HRP Dilution Buffer.
   c. Vortex the vial until the Streptavidin-HRP is thoroughly mixed.
   d. Store unused Streptavidin-HRP at 4°C. Good for 1 week.

5) Wash Buffer
   a. Place 50 ml of the 20X Wash Buffer in a clean sterile 1-Liter bottle. Bring up to 1 Liter with deionized water.
   b. Invert bottle to insure sufficient mixing.
   c. Store unused wash buffer at 4°C. Good for 1 year.

6) Substrate A & Substrate B
   a. Once the components of Substrate A & Substrate B are mixed, they can be stored at 4°C and will be stable for 4 months.

7) 96-Well Stripwell Microplate
   a. Once the 96-well stripwell microplate is opened, the unused stripwells must be covered with a plate seal and sealed in the Mylar Ziplock bag provided to ensure
Procedure

Note: *It is recommended that reagents be warmed to room temperature before they are added to the plate, with the exception of the Wash Buffer, which should be kept at 4°C.*

1) Six Point Standard Curve Preparation
   a. For duplicate standard curves, you will need a total of 60 µl of each point on the standard curve.
   b. Prepare standards by transferring a minimum of 65 µl from the Antigen Standard to a separate tube containing 65 µl of Sample Dilution Buffer and mix thoroughly. This will be the 1:2 point on the standard curve.
   c. Make the 1:4 point for the standard curve by removing 65 µl from the 1:2 dilution and adding it to 65 µl of Sample Dilution Buffer in a separate vial.
   d. Carry out these 1:2 serial dilutions two more steps until the last point is at a final dilution factor of 1:16.

   ![Diagram of standard curve preparation](image)

   Note: The additional 5 µl is to insure there is enough reagent for each point of the standard curve.

2) Sample Preparation
   a. All mouse samples must be diluted with Sample Dilution Buffer to prevent interference from mouse serum.
   b. If you anticipate that your sample concentration will be higher than the ranges on the standard curves, use the Sample Dilution Buffer to dilute your samples.
   c. Various sample types may be tested with this kit such as tissue culture supernate, serum, plasma and urine. This kit has also been tested for inhibitors such as NP40, Tween, SDS and Heparin. For a complete listing with more information, please see Appendix B.
3) Addition of Samples to the 96-Well Stripwell Microplate

a. Place desired number of stripwells into the holder. If you choose not to run the entire plate at this time, make sure the unused stripwells are removed from the tray, covered with a plate seal and placed in the Mylar Ziplock bag provided to ensure that the stripwells do not dry out.

b. The standard curve can be placed anywhere on the plate, however, in accordance to the plate diagram below, the process of the standard curve addition will be explained.

c. Place 30 µl of the Antigen Standard in the first wells of the rows (G1 and H1). These wells also serve as a positive control in that they should always have a bright response. In the following rows (G2-G5 and H2-H5) place 30 µl from each of the dilutions done in step 1. In the last well (G6 and H6) place 30 µl of the Sample Dilution Buffer. This well will be used to determine the background of the assay and also serve as a negative control.

d. In each well place 30 µl of sample into its appropriate location. (See diagram below to plan the location of samples.)

e. Once the standard curve and samples have been added to the plate, place the plate on an orbital shaker at 120 RPM for 1 hour at room temperature.

f. The remaining reagents can be stored at 4°C for 1 week except for the Antigen Standard. It must be frozen after reconstitution and used within one week.

Note: Make sure you add samples and standard curve in a timely manner. Do not take longer than 10 minutes to add them to the plate. To help, you may want to prepare them in a different plate and transfer when ready.

Caution: Do not touch the pipette tips on the side of the well when adding sample or standard curve.
Procedure cont.

4) Washing of the Plate
   a. Using the predetermined wash method, wash the plate three times.
      (For more information, see Appendix A)

5) Addition of Detection Mix
   a. Add 30 µl of the previously reconstituted Detection Mix to each of the
      wells that received sample, whether it was the standard curve,
      control, or unknown samples.
   b. Place the plate back on the orbital shaker at 120 RPM for 1 hour at
      room temperature.

   Caution: Do not touch the pipette tips on the side of the well when adding
   Detection Mix.

6) Removal and washing of the Detection Mix from the Plate
   a. Using the predetermined wash method, wash the plate three times.

7) Addition of Streptavidin-HRP to the Plate
   a. Add 30 µl of the previously diluted Streptavidin-HRP to each of the
      wells that received sample, whether it was the standard curve or
      unknown samples.
   b. Place the plate back on the orbital shaker at 120 RPM for 15 minutes
      at room temperature.
   c. In preparation for imaging the plate, mix the contents of Substrate A
      with those of Substrate B. Allow the mixed Substrate to sit at room
      temperature while the Streptavidin-HRP incubation takes place.

   Caution: Do not touch the pipette tips on the side of the well when adding
   Streptavidin-HRP.

8) Removal and washing of the Streptavidin-HRP from the Plate
   a. Using the predetermined wash method, wash the plate six times.

9) Addition of Substrate and Imaging of the Plate
   a. Ensure that the previously mixed Substrate is at room temperature
      before addition to the plate.
   b. Add 40µl of the Substrate to each well.
   c. Image the plate immediately after Substrate is added for optimal
      results. Wait no longer than 15 minutes to commence imaging.
   d. Place the plate in the imager and allow the Substrate to incubate in
      the wells for 30 seconds prior to imaging the plate.
Procedure cont.

Caution: Do not touch the pipette tips on the side of the well when adding Substrate. The plate should be handled gently after addition of substrate with no bumping or vibration.

10) CCD Imaging of the Plate
    a. With the CCD camera set up, image the plate at 1 minute and 4 minute intervals. Exposure time will vary from the guidelines, depending on the type of camera used. If the image is too bright at the 1 minute interval, decrease the exposure time. If it is too dim at the 4 minute interval, increase the exposure time until the image looks acceptable.

    Note: These instructions are for Cooled CCD cameras. See page 14 for Non-Cooled and Low Resolution camera recommendations.

    b. Use the available software to analyze the array. (See step 11)

Caution:
1) Make sure the imager is light tight. Any light leaking in during imaging will ruin the image.
2) This is a chemiluminescent reaction. It does not require an excitation source. If your plate is exposed to UV light, or another source, it will not work.

11) Quansys Array Software
    a. Please visit www.quansysbio.com to download a free copy of the Quansys Array Software.

12) Additional Services
    Please contact Technical Support at 1-888-782-6797.
Imager Information

To ensure an optimal response from your Q-Plex™ Array, review the following information on cooled, non-cooled, and low-resolution CCD imagers.

Cooled CCD:
If the camera is cooled, image the plate at 1 minute and 4 minute intervals. Exposure times may vary from the guidelines, depending on the type of camera used. If the image is too bright at the 1 minute interval, decrease the exposure time. If it is too dim at the 4 minute interval, increase the exposure time until the image looks acceptable (clear and defined spots). Make sure that the top two points on the standard curve are not saturated. There must be a 10-20% pixel intensity difference between the high point (Antigen Standard) and the 1:2 point on the standard curve.

Non-Cooled CCD:
If the camera is non-cooled, we recommend imaging for one minute or less. Check with your manufacturer to determine maximum exposure times. CCD cameras are cooled to allow long exposure times without damaging the CCD chip.

Low-Resolution CCD:
If the camera has low resolution, <1 Megapixel, take multiple pictures of the plate. Zoom your camera in to each ¼ to ½ of the plate. Place the standard curve on each portion of the plate, one standard curve on columns 1-6 and another on 7-12.

Note: It is optimal to image your plate immediately after Substrate is added.

For more details on your CCD imaging system, please visit our website at www.quansysbio.com or contact Technical Support at 1-888-782-6797.
Appendix A

1) Automatic Stripwell Microplate Washer
   a. Connect the prepared wash buffer to your automatic plate washer.
   b. Run 1-2 prime cycles to make sure that the wash buffer is running through the plate washer. (When the buffer has run through the machine, the waste will be foamy.)
   c. Make sure that the plate washer is able to wash a stripwell microplate (dispense and aspirate 300 µl of wash buffer) three times and six times. Both types of washes are used in the protocol.
   d. No soak or shaking cycles are needed.
   e. Prime the plate washer one time before each wash step.
   f. Inspect the plate for residual wash solution. If residual wash solution remains, vigorously tap the upside-down plate against a paper towel on a hard surface to remove any excess wash solution.

2) Multichannel Pipette
   a. After each incubation, aggressively flick the solution out of the plate over a waste container before starting the wash protocol.
   b. Pour the prepared Wash Buffer into a trough or tray.
   c. Using a multichannel pipettor, pipette 200-300 µl of Wash Buffer into each of the wells used in the test.
   d. Aggressively flick the wash buffer out over a waste container.
   e. This washes the plate one time.
   f. Inspect the plate for residual wash solution. If there is any, get a paper towel, set it on the counter and tap the plate upside down against the paper towel to remove any

Appendix B

Interferences and Compatibility

Inhibitors:

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Interference at</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP40</td>
<td>1%</td>
</tr>
<tr>
<td>Tween</td>
<td>1%</td>
</tr>
<tr>
<td>Triton</td>
<td>1%</td>
</tr>
<tr>
<td>Citrate</td>
<td>20%</td>
</tr>
<tr>
<td>SDS</td>
<td>Interfered with Assay</td>
</tr>
<tr>
<td>EDTA</td>
<td>20 mM</td>
</tr>
<tr>
<td>Water</td>
<td>55 M</td>
</tr>
<tr>
<td>Heparin</td>
<td>30 mg/ml</td>
</tr>
<tr>
<td>Urea</td>
<td>1 M</td>
</tr>
<tr>
<td>DMEM</td>
<td>100%</td>
</tr>
<tr>
<td>HAMS</td>
<td>100%</td>
</tr>
<tr>
<td>RPMI</td>
<td>100%</td>
</tr>
<tr>
<td>SFM4</td>
<td>100%</td>
</tr>
</tbody>
</table>

Tissue culture supernatant, serum, plasma, urine, cell extracts, and tissue homogenates are compatible with the assay.
Troubleshooting

1) No Response
   a. Did the positive control light up?
      1) No - Streptavidin-HRP is bad and did not work
      2) Yes
   b. Did you add sample?
      Review the procedure steps and see if anything was overlooked.
      1) Incubation times
      2) Correct buffers used
      3) Correct reagents used

2) Low or Saturated Response
   a. Low Response
      1) Did you use the proper volume to reconstitute the detection mix? If you used too much water, then they will be too dilute and produce a low response.
      2) Did you dilute the samples too much? Diluting them further than the recommended amount may produce a low response.
      3) Did you wash the plates more than was recommended? Too many washes may diminish the response.
      4) Have you used this plate in a previous test where the blank wells received a wash? This may prematurely diminish the response because the capture antibodies received extra washes.
      5) Did you use the proper amounts of substrate solution A and B? If not mixed together at equal volume the response may be diminished.
      6) How long did you image your plate? Too short an exposure time will produce a low response.
   b. Saturated Response
      1) How long did you image your plate? Too long an exposure time will produce a saturated response.
      2) Did you use the proper volume when mixing the Streptavidin-HRP? If you add too much Streptavidin-HRP Concentrate, then the mix will be concentrated and produce a saturated response.
      3) Did you use the proper dilutions for your samples? If your samples are too concentrated, you will get a saturated response.

3) Background
   a. Have the kit reagents expired? Old reagents may create a higher background.
   b. Did you use the buffer supplied with the kit to dilute your samples? The assay has been optimized to use the buffers supplied with the kit.
   c. Were the kit reagents left at a temperature other than 4°C for an extended amount of time? If so, the reagents may degrade and cause high background.
   d. If every well has high background, the substrate may be contaminated with Streptavidin-HRP.
   e. Inadequate washing. Too few repetitions.
Troubleshooting cont.

4) Poor Standard Curve
   a. Were the dilutions for the standard curve made correctly? Double-check all of your calculations to make sure there was no human error.
   b. Pipettor error-miscalibrated or malfunctioning pipettor

5) Replicate Variability
   a. If the replicates do not show an identical profile (the way the spots light up), then the samples may have been placed in the wrong wells.

6) Spot Clarity Issue
   a. If you notice comet tails on your spots (bright spots that resemble the shape of a comet-blurring or bleeding into another spot), make sure that you move your plate to the imager very carefully. Try not to rock or jiggle the plate when moving it to the imager, because doing so can blur the spots.
   b. Insure that the washing solution is at 4°C.

7) Random Luminescence
   a. If a crescent-shaped luminance shows up in some of the wells, then most likely you touched the pipette tips to the side of the well when adding Streptavidin-HRP and substrate.
   b. Check your automatic plate washer to see if any of the channels are plugged.
   c. If washing by hand, make sure that you completely fill each of the wells with wash solution.
   d. If washing by hand, make sure that you flick out the excess wash solution very well.

8) Vignetting
   a. If you notice a difference in the response of the same sample from the left side of the plate to the right side of the plate, you may have vignetting. This is an optical problem that arises from using a camera to image the plate. The camera lens does not capture light traveling from the plate on the outside wells the same way that it captures light traveling from the plate on the middle wells.
   b. To fix this problem, you need to make sure that you are using the recommended camera and lens to image the plates. Also, make sure that you have the camera mounted at the recommended height away from the plate.
   c. You may need to try taking a few up-close pictures (moving the camera closer to the plate) to see if it fixes the problem.

9) Failed Control
   a. Positive Control
      1) Did you add Antigen Standard, Detection Mix, and Streptavidin-HRP to the positive control wells or the top point of the standard curve before adding substrate to the wells and imaging the plate?
   b. Negative Control
      1) If the negative control lights up, then your substrate is contaminated with Streptavidin-HRP. You will have to test the stock vials of the substrate to insure that they are not also contaminated.
      2) If the plate is not properly washed, residual Streptavidin-HRP will remain in the wells and cause the negative control to light up.
Abbreviated Protocol

1) Set up the camera using the included mouse pad as a guide.

2) Determine the method of plate washing and prepare the wash buffer.

3) Reconstitute the lyophilized vials by adding the indicated amount of dH₂O.

4) Prepare Streptavidin-HRP by adding 2 µl of Streptavidin-HRP Concentrate to the Streptaividn-HRP Dilution Buffer vial.

5) Prepare the standard curve by doing 1:2 serial dilutions of the Antigen Standard with the Sample Dilution Buffer.

6) Prepare the samples.

7) Add the standard curve to the bottom left hand corner of the plate in rows G & H.

8) Add the samples to the plate.

9) Incubate the samples and standard curve for 1 hour.

10) Wash the plate 3 times.

11) Add the Detection Mix to every well of the plate used in the test.

12) Incubate the Detection Mix for 1 hour.

13) Wash the plate 3 times.

14) Add the Streptavidin-HRP to every well of the plate used in the test.

15) Incubate the Streptavidin-HRP for 15 minutes.

16) Mix Substrate A and Substrate B together and allow to sit at room temp.

17) Wash the plate 6 times.

18) Add the Substrate to every well of the plate used in the test.

19) Image the plate at 1 and 4 minute intervals.