Table of Contents

At R&D Systems, we strive to provide you with quality immunoassays that guarantee the utmost performance.

Our development and QC programs are designed to assure the highest quality immunoassays. Each kit is put through rigorous validation and stability testing to ensure precision, accuracy, sensitivity, specificity, and that results obtained are reproducible for the life of the kit and from lot to lot.

Development Program

- Careful selection of antibody pairs for optimal performance
- Coated microplates with precision of less than 10% CV (coefficient of variation)
- Cross-reactivity and interference testing with a panel of up to 100 factors
- Determination of sensitivity
- Specially formulated diluents that alleviate interferences due to matrix phenomena and heterophilic antibody interactions
- Correlation to NIBSC/WHO Standards when available
- Optimization of performance with all sample types

Quality Control Program

- Intra- and Inter-assay precision must have a CV that is typically less than 10%
- Controls must meet established specifications
- NSB (non-specific binding), low standard, and high standard signals must meet the established specifications
- Sensitivity, determined by assaying multiple replicates of the zero standard, falls within the established specifications
- Standards must match Master Calibrators within the established specification

We have designed this troubleshooting booklet in addition to the kit insert to help guide you through any questions you may have. If you have questions regarding a specific assay, please contact Technical Service at 1-800-343-7475 in the U.S.A. or +44 (0)1235 551100 in Europe and a representative will be happy to assist you.

Immunoassay failure can be attributed to many factors. Most technical errors can be avoided if the kit insert is read and fully understood before starting the assay. If there are any questions regarding the kit insert, please call Biotech Technical Service at 1-800-343-7475 in the U.S.A. or +44 (0)1235 551100 in Europe before you run the assay.

- Check expiration date of the kit. The kit should not be used if it is past the expiration date specified on the kit label.
- Check storage conditions of individual components and ensure that all reagents have been stored as indicated in the kit insert.
- Check for signs of instability or deterioration in reagent solutions (*e.g.*, precipitation or discoloration). Some reagents, such as Calibrator Diluents or Assay Diluents, may contain a precipitate by design. Mix all reagents thoroughly before adding to the plate. Depending on the nature of the precipitate, it may be necessary to mix continually while adding to the plate.
- Ensure that specified incubation times and temperatures are adhered to.
- Do not substitute kit reagents or mix reagents from different kit lot numbers.
- When mixing or reconstituting protein solutions, always avoid foaming.
- Determine what samples will be assayed and preserve the integrity of the samples by using aseptic technique.
- Document the assay layout before you start the assay. A plate layout is provided on page 23.
- Obtain all equipment required (*e.g.*, pipettes, tips, test tubes, washer, plate reader) before beginning the assay.
- Clean the benchtop on which the assay is to be run.
- If controls are provided in the kit, always be sure to run the controls with each assay. The concentrations of the controls should fall within the specified range.
- If controls are not provided in the kit, commercial controls are available for most analytes. Contact Technical Service.
- Due to limitations of many luminometers, it is recommended that the standards be assayed in duplicate from high to low, beginning with the high standard in wells A1 and A2.

Correct washing of the plate is a very critical and important aspect of running any successful ELISA. Consistency in washing the plate is essential. When diluting the Wash Buffer Concentrate, use deionized or distilled water.

- **Squirt bottle or multi-channel pipette -**

  Ensure that the wash bottle has good pressure or that each prong of the pipette is dispensing properly and is free of debris. Check the strips in the plate so that they are in the plate holder securely. Numbering the strips may be useful in the event that strips become loose while decanting. First, empty the contents of the plate by decanting. Fill each well with the volume of Wash Buffer specified in the kit insert. If the assay protocol calls for a soak time, set a timer for the full amount of time and let the Wash Buffer soak in the wells. Decant the wells by inverting the plate and blotting it against clean paper toweling. Repeat this process as directed in the kit insert. After the last decanting, remove any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean paper toweling on a hard surface. Do not allow the wells to sit dry. Proceed immediately to the next step in the kit insert.

- **Manifold dispenser or autowasher -**

  Hook up the dispenser or autowasher to an appropriate vacuum supply (refer to manufacturer's guidelines). Ensure that each cannulae or prong is dispensing and aspirating properly. First, empty the contents of the plate by aspirating or decanting. Fill each well with the volume of Wash Buffer specified in the kit insert. If the assay protocol calls for a soak time, set a timer for the full amount of time and let the Wash Buffer soak in the wells. Aspirate the wells completely, ensuring that no Wash Buffer is left in the wells. Do not over-aspirate the wells by allowing the aspiration apparatus to sit in the wells after the liquid has been aspirated. Repeat this process as directed in the kit insert. After the last aspiration, remove any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean paper toweling on a hard surface. Do not allow the wells to sit dry. Proceed immediately to the next step in the kit insert.

Washing the plate too rapidly or too slowly, incomplete washing or aspirating, and allowing the wells to sit dry are all factors that will affect the precision of the assay.

Ensure that all pipettes used are properly calibrated. To check the calibration, pipette 10 replicates of deionized water at the minimum volume of the pipette (as guaranteed by the manufacturer). The CV of the 10 replicates should be less than 2-3%. Repeat the process using the maximum volume of the pipette. If the CVs of either test are greater than 2-3%, repeat the procedure. If the CVs remain above 2-3%, the pipette needs to be repaired before running the assay.

♦ Forward mode pipetting -

Set the pipette to the desired volume. Pre-rinse the pipette tip with the reagent or sample. Depress the plunger to the first stop. Draw up the liquid slowly, allowing the plunger to return to the up position. Never let the plunger snap up. Wait a moment to allow the liquid to reach volumetric equilibrium in the tip. Dispense the liquid to the first stop and then gently to the second stop. Hold the plunger at this stop until the pipette tip is removed from the reservoir/well to avoid drawing up the liquid. As you remove the pipette tip from the reservoir/well, slide the tip on the side of the reservoir/well to release any liquid that may be on the outside of the tip.

♦ Reverse mode pipetting -

To reduce error due to surface tension of the liquid, this method is often recommended. Set the pipette to the desired volume. Pre-rinse the pipette tip with the reagent or sample. Depress the plunger all the way to the second stop. Draw up the liquid slowly, allowing the plunger to return to the up position. Never let the plunger snap up. Wait a moment to allow the liquid to reach volumetric equilibrium in the tip. Dispense the liquid only to the first stop. Hold the plunger at this stop until the pipette tip is removed from the reservoir/well to avoid drawing up the liquid. As you remove the tip from the reservoir/well, slide the tip on the side of the reservoir/well to release any liquid that may be on the outside of the tip.

Pipetting Tips

- The key to repeated accuracy in pipetting is a consistent technique. Consistent speed and smoothness while pipetting is necessary. Avoid sudden motions when drawing or dispensing fluids.
- Check to be sure that the pipette tip is fitted properly on the end of the pipette and seated with adequate pressure.
- Change pipette tips between each sample or reagent addition.
- Do not pipette a lower volume than is suggested by the pipette manufacturer. This will decrease accuracy and reproducibility.
- Some liquids will have a tendency to cling to the inside or outside surfaces of the pipette tips. To avoid variations in the volumes drawn or dispensed caused by this surface tension, pre-rinse the pipette tip with the sample or reagent. This will improve accuracy when pipetting.
- If the reagent to be pipetted is viscous, wait a moment after drawing the liquid to allow the volume to reach equilibrium before removing the pipette tip from the reservoir.
- After drawing the liquid into the tip, remove the tip from the solution and wipe the outside of the tip with a lint-free tissue.
- If an air bubble appears in the tip while drawing the liquid, dispense the liquid back into the container and re-pipette the liquid at a slower rate. If the air bubble appears a second time, discard the pipette tip and replace with a new tip.
- If foaming occurs in the tip while drawing up the liquid, it may be necessary to hold the pipette at a slight angle and draw the liquid slower.
- Use separate reservoirs for each reagent.

1 Storage

Can I collect my samples prior to the day I run my assay?

- Yes, unless otherwise indicated by the kit insert. If samples cannot be assayed immediately, aliquot and store samples at ≤-20° C or as directed in the kit insert. Always avoid repeated freeze-thaw cycles.
- For ≤-20° C storage, it is recommended that a set temperature freezer be used. The warm-up cycle of a frost-free freezer may cause degradation of samples.

2 Preparation

Do I have to use the sample preparation method in the kit insert?

- Recommended sample preparations have been tested for optimal recovery and performance in each assay. It is recommended that the preparations be carried out as outlined in the kit insert.

Can I use a plasma collection other than the recommended collection?

- Each assay has been validated using the outlined procedure. Certain anticoagulants are not recommended in some assays. Refer to the kit insert.
- Some analytes are present in platelets, therefore, platelet-poor plasma is recommended. Refer to the kit insert for the proper collection procedures.

Can I use my own diluents/buffers?

- Each kit contains diluents that are formulated to closely match specific sample types. Ensure that the correct diluent is used for the sample type being tested. Refer to the kit insert.

Helpful Hints

- Determine what samples will be run and use aseptic technique during collection and preparation.
- If several samples are to be run, keep samples separate and clearly labeled to avoid cross-contamination.
- Prior to assay, centrifuge samples to get rid of particulates. Transfer each sample to a fresh tube and mix to assure homogenity.
- When mixing samples, use a tube that has a volume capacity that is at least 50% greater than the sample volume so adequate mixing can occur.

2 Preparation Cont.

I don't have enough diluent to run all my samples. What do I do?

- Enough diluent is provided if the protocol is followed. It is assumed that all standards and samples are run in duplicate.
- If no dilution is suggested, or a greater dilution is required, calculate the amount of diluent needed prior to setting up the assay. Additional diluent can be obtained by contacting Technical Service.

3 Misc.

Can I use a sample type that is not recommended in the kit insert?

- The kit has been validated for the sample types listed in the kit insert. Sample types other than those validated have not been tested.
- Contact Technical Service for further information.

4 Sample Values

I ran controls but they were out of range. What does this mean?

- If controls were run, the concentration of the control must be within the range specified. If the control values were outside the range, the assay may be invalid. Refer to pages 12-14.
- Be sure controls are reconstituted properly.

Can I use the sample data in the kit insert as a reference?

- The sample data provided in the kit insert has been generated from a very limited population of individuals and should be used as a general guideline only.

Can I read my samples off the standard curve in the insert?

- No. Sample values must be determined from the standard curve generated in each assay.

How do I determine my sample concentration from a hand-plotted standard curve?

- To determine the concentration of each sample, first find the absorbance value or relative light unit (RLU) on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration.

4 Sample Values Cont.

How do I compensate for dilution or concentration of my samples?

- If samples were diluted, the value read from the standard curve must be multiplied by the dilution factor.
- If samples were concentrated, the value read from the standard curve must be divided by the concentration factor.

How do I convert my sample value to Units?

- If the NIBSC/WHO International Standard was available, there will be a conversion equation in the Calibration section of the kit insert. Plug your sample value into the equation to convert pg/mL or ng/mL to Units.

My samples generated values that were outside the dynamic range of the assay. Can I use these values?

- It is recommended that only sample values that fall within the range of the standard curve be used. Values outside the range of the standard curve are generally non-linear, which can lead to incorrectly extrapolated values.
- Samples that generate values higher than the highest standard should be (further) diluted and the assay repeated.
- If samples fall below the range of the assay, the sample is considered to be non-detectable.

My standard curve looked fine, but I didn't get a signal in my sample when I expected to, why?

- The sample may not contain the analyte.
- A matrix effect may be masking the detection. Ensure that the recommended dilution was followed as stated in the kit insert.
- If dilution was recommended, check to be sure that the dilution was performed properly. Over-dilution may cause the sample to fall below the range of the standard curve.

Immunoassay precision is defined as the reproducibility between wells within an assay (intra-assay) and between assays (inter-assay). Poor precision, determined by high CVs, is often caused by 2 main factors: pipetting technique and washing technique.

1 Pipetting Technique

Do I need to mix reagents in the well?

- Yes, when multiple reagents are pipetted into one well (*e.g.*, assay diluent and sample). Pipette reagents and samples into the center of each well. Tap the plate gently to ensure mixing of reagents.

My sample/reagent is viscous and isn't being pipetted properly. What can I do?

- Wait a moment to allow the volume to reach equilibrium before removing the pipette tip from the reservoir.
- Pre-rinse the pipette tip with the reagent to compensate for surface tension.

Pipetting Hints

- Inadequate or uneven fill volumes in the wells is a good indication that the pipette needs to be calibrated. Check the pipette function and recalibrate if needed.
- Always be sure to change pipette tips between reagents, standards, and samples to avoid carryover or cross-contamination.
- Check to be sure that the pipette tips are on completely. A pipette tip that is not fitted properly may not draw or dispense the volume accurately.
- As you remove the tip from the well, slide the tip on the edge of the well to remove any liquid remaining on the outside of the tip. Refer to Pipetting Techniques on pages 5 and 6.

2 Washing Technique

Helpful Washing Hints

- If using an automated washer, aspiration apparatus, or a multi-channel pipette, always be sure that each cannulae (prong) is dispensing and aspirating properly. After the last wash, decant any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean paper toweling on a hard surface. Refer to Washing Techniques on page 4.

2

Washing Technique Cont.

- Washing the wells too rapidly or slowly may result in poor precision.
- Do not allow the wells to sit dry. Proceed immediately to the next step in the kit insert.

Can I use less Wash Buffer?

- Using less Wash Buffer will often lead to poor CVs. Be sure to wash the wells with the volume specified in the kit insert to ensure that each well is thoroughly washed.

I ran out of Wash Buffer. Can I use Wash Buffer from another kit?

- Not all Wash Buffers are identical. Call Technical Service for assistance.

Can I skip the soak time in the wash step?

- Some assays recommend a soak between washes. Skipping this step, or shortening the soak time can lead to poor precision. Always follow the directions in the kit insert for optimal performance.

Can I cut down on the number of washes?

- It is recommended that the number of washes specified in the kit insert be adhered to. Changing the number of washes can affect the precision of the assay.

3

Misc.

Can I use the same reservoir to pipette all of my reagents?

- No. Use separate reservoirs for each reagent to avoid contamination.
- Some analytes are highly susceptible to contamination (*e.g.*, saliva, oxidizing reagents, etc.). Refer to the kit insert. Take necessary precautions to protect kit reagents.

Can I reuse the plate sealer?

- A reused plate sealer may contain residual from the previous step which can contaminate the current component in the well, leading to poor precision. Use a new plate sealer for each incubation as recommended in the kit insert.

Standard curve results can be affected by many factors, including preparation and technique.

1 Preparation

Can I alter my standard preparation?

- No. Reconstitute the standard with the appropriate volume of Calibrator Diluent or deionized water as directed in the kit insert.
- When mixing or reconstituting standards, always avoid foaming.
- Always allow standard to sit for a minimum of 15 minutes (refer to kit insert) after reconstitution. Mix gently before use. Assure that all solids have dissolved before using.
- When preparing the standard curve, ensure accurate completion of the dilution series. Always change pipette tips between dilutions. Mix dilutions thoroughly before the next transfer.

Can I dilute the standard in other media instead of the Calibrator Diluent?

- Other media may contain substances that can affect the results of the standard curve. Media can be run as a sample control.
- When reconstituting the standard or preparing the standard curve, use the Calibrator Diluent that most closely represents the sample type. Refer to the kit insert.

2 Technique

How does washing affect my standard curve?

- Incomplete washing/aspiration of wells can cause poor precision, which may yield poor standard curve results. Ensure that the washing apparatus is working correctly and that the wells have been aspirated thoroughly without allowing them to sit dry. Refer to Washing Techniques on page 4.

How does pipetting affect my standard curve?

- Inconsistent pipetting can cause poor replicates between wells leading to high CVs and a poor standard curve.
- An uncalibrated pipette can cause dilutional errors during the standard curve preparation which can cause a shift in the standard curve and/or a non-linear curve. Ensure that the pipette is functioning properly. Unequal volumes between wells is an indication of possible pipette malfunction or incorrect application of pipette tip. Refer to Pipetting Techniques on pages 5 and 6.

3

Misc.

My computer software is not capable of the recommended data reduction. Can I use a different one?

- Yes, however, each assay has been optimized using the data reduction specified in the assay protocol. The use of a different data reduction method may cause a shift in results.
- As an alternative to computer software, plot the optical density for the standards on the y-axis versus the concentration of the standards on the x-axis. The data may be linearized by using log/log paper and regression analysis may be applied to the log transformation. If log/log paper is not available, linearize the data by plotting the log of the concentrations versus the log of the OD. The best fit line can be determined by regression analysis.

How do I determine my sample concentration from my hand-plotted graph?

- To determine the concentration of each sample, first find the absorbance value or RLU on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor

Can I use the standard curve in the insert to read my samples?

- No. Standard curves shown in the kit insert are representative of typical results. A standard curve run in duplicate should be generated for each set of samples assayed.

Can I change the incubation times according to my schedule?

- Altering incubation times or temperatures can prevent the assay from reaching equilibrium or can over-develop the assay which may lead to a change in the slope of the curve. Always adhere to the recommended incubation times and temperatures.

3 Misc. Cont.

Can one standard curve be run when assaying multiple plates?

- When running samples on multiple plates, a standard curve should be run on each plate. Plate-to-plate variability often occurs due to technical errors or differing incubation/environmental conditions between plates. Samples must be determined from a standard curve assayed under the same conditions or the values may be invalid.

My standard curve was flat or non-linear. What may have happened? Many factors can cause a standard curve to be flat. The following factors tend to be the most common.

- Ensure that appropriate dilution series was followed. Refer to the kit insert.
- Ensure that correct wavelength and wavelength correction was used to read the plate.
- Standards and samples should be added to the plate within 15 or 20 minutes (unless indicated otherwise in the kit insert).
- Check to see if backgrounds are high.
- Ensure that the plate was washed correctly. Inadequate washing may cause a flat standard curve with a very high signal. Refer to Washing Techniques on page 4.
- Always read the plate within the reading window indicated in the assay protocol. Reading a plate beyond the suggested reading times may lead to erroneous results.
- If the highest standard is reading above the limits of the plate reader, ensure accurate reconstitution of standard and that all incubation times and temperatures have been adhered to.
- Always run standards in duplicate to ensure accuracy.
- If controls were run, the concentration of the control must be within the range specified.
- Ensure that reagents weren't contaminated during the assay set-up or incubation. Refer to pages 3, 11, and 18.
- A flat standard curve with very little signal may be an indication that the conjugate enzyme or substrate isn't performing properly. To test the performance of these components, refer to page 19.
- Due to limitations of many luminometers, it is recommended that the standards be assayed in duplicate from high to low, beginning with the high standard in wells A1 and A2.

Edge effect is defined as an obvious difference in the signal of the outer wells of the plate compared to the signal of the inner wells and is commonly attributed to two main factors.

1 Temp.

Does it really matter at what conditions I incubate my plate?

- Yes. Incubating your plate where there are uneven temperatures around the work surface can cause an edge effect. Always adhere to recommended incubation temperatures. Avoid running the assay in an area where the temperature tends to vary greatly. If available, incubate the plate in a controlled environment such as an incubator.
- Bring all reagents to room temperature before use (unless otherwise specified). Refer to the kit insert.
- Observe the temperature of the benchtop on which the assay is to be run. If the curve is suppressed, the benchtop may be much colder than the air temperature.

2 Plate Sealers

At the end of the incubation period, I noticed that the plate sealer had lifted on one side. How will this affect my assay results?

- Plate sealers that are not sealed properly can lead to an edge effect. The outer wells that are uncovered may be exposed to environmental conditions such as air flow and temperature, contamination of the conjugate or substrate, or evaporation of reagents. Always be sure to adhere the plate sealer properly by firmly pressing the sealer along the edges of the plate.

Drift is defined as a noticeable change in signal from either the left side to the right side of the plate or from the top to the bottom of the plate.

1 Assay Set Up

I started to set up my assay and was interrupted. How will this affect my results?

- Interruption in the set up of an assay can lead to a drift pattern on the plate. Be sure to have all standards and samples prepared before you begin pipetting. Always pipette the reagents onto the plate within 15 or 20 minutes (unless otherwise indicated in the kit insert).

Do I need a multi-channel pipette?

- When dispensing reagents such as Substrate, Conjugate, or Assay Diluents, a repeater or multi-channel pipette is preferred.

2 Reagents

What happens if I don't warm up my reagents?

- Cold reagents can lead to temperature differences between wells which can cause a variation in signal across the plate. Reagents should always be warmed to room temperature before use unless otherwise indicated in the kit insert.
- Each assay has been optimized at the indicated incubation times and temperatures. Cold reagents will commonly require an increase in the length of incubation time. Always adhere to the recommended incubation times and temperatures.

3 Plate Sealers

Do I need to use the plate sealers?

- Refer to kit insert for instructions. If the assay protocol recommends using the plate sealer, ensure that the plate sealer is adhered to the plate properly by firmly pressing the sealer along the edges of the plate. If the plate sealer has lifted on one side of the plate, a difference in signal may be seen.

4 Read Time

How do luminometer settings affect my results?

- For chemiluminescent assays, reading at greater than or equal to 2.0 seconds/well will produce a drift because of the time elapsed from the first wells read to the last wells read. During the substrate reaction, horseradish peroxidase is recycled until there is no more substrate available. As time elapses, less substrate is available and light production will be affected. Set the luminometer to a 1.0 sec/well read time.

5 Incubation

Can I alter incubation times to fit my schedule?

- Altering incubation times or temperatures can prevent the assay from reaching equilibrium or can over-develop the assay. Always adhere to the recommended incubation times and temperatures.
- If multiple assays are being run simultaneously, time each plate individually to avoid over- or under-incubating a plate.

Signal development can be affected by several factors: substrate, incubation, conjugate or substrate failure, wavelength, and instruments.

1 Substrate

Can I alter the substrate preparation?

- If substrate solution requires mixing, ensure that the correct volume of each substrate reagent is added and mixed thoroughly. Mixed substrate solution should be used within 15 minutes (up to 4 hours for chemiluminescent assays). If substrate is mixed too early, color may begin to develop in the reservoir.
- If substrate is lyophilized or tablets are used, dissolve completely with the appropriate volume of diluent as indicated in the kit insert. Mix thoroughly and use within the time indicated in the kit insert.

Do I need to pipette my substrate in any particular order?

- Reagents should always be pipetted in the same order throughout the assay.
- High sensitivity assays utilize an amplification system. Amplifier must be added to the wells after the substrate incubation and pipetted in the same order as the substrate.
- After the substrate has been pipetted into the wells, tap the plate gently to mix thoroughly.

Is it possible to contaminate the Substrate?

- Yes. If the assay uses an alkaline phosphatase or horseradish peroxidase labelled conjugate, care must be taken to avoid contamination of substrate during the assay. Contamination of the substrate may yield a value that exceeds the limitations of the plate reader. Avoid contact with metals or oxidizing reagents. Use a new reagent reservoir.

Do I have to incubate my Substrate in the dark?

- No, but avoid placing the plate in direct light during the substrate incubation.

2 Incubation

Can I alter the incubation?

- Each assay has been optimized at the indicated temperatures and incubation times. Varying the incubation times and temperatures may lead to inadequate or too high a signal development.
- If multiple assays are being run simultaneously, time each plate individually to avoid over- or under-incubating a plate.

How does temperature affect my signal?

- Both alkaline phosphatase and horseradish peroxidase are temperature-sensitive enzymes. Optical Density (OD) units or relative light units (RLU) may vary with temperature changes.
- Avoid incubating plate in areas where environmental conditions vary greatly (*e.g.,* under heat or air vent, on window sill where temperature and light may vary throughout the assay).

---

3 Conjugate or Substrate Failure

To ensure that conjugate enzymes and substrate are performing properly:

- For colorimetric assays -

  Mix equal volumes of conjugate and substrate solution (for high sensitivity kits, add equal volumes of conjugate and substrate. After 1 minute add an equal volume of amplifier). Color should develop immediately. If color does not develop, check to ensure components have been stored at the correct storage temperatures, are not contaminated, and are within the expiration dates.

- For chemiluminescent assays -

  Add 10 µL of conjugate to 190 µL of substrate. Check light production using a luminometer. If light is not produced, check that the components have been stored at the correct storage temperatures, are not contaminated, and are within the expiration dates.

4 Wavelength

Do I need to use wavelength correction?

- For colorimetric assays, Yes. This will correct for optical imperfections in the plate.

What can I do if my plate reader does not have wavelength correction available?

- Read the plate at both wavelengths and subtract the wavelength correction readings from the primary wavelength readings. This subtraction will correct for optical imperfections in the plate.

My plate reader does not have the same filter as the recommended correction wavelength. Can I substitute?

- Yes, as long as the wavelength chosen is higher than the recommended correction wavelength.

My plate reader does not have the suggested primary wavelength filter. Can I substitute?

- Primary reading wavelength is determined by the substrate used and peak absorbance of its resulting color. Always adhere to the recommended wavelengths. If not available, choose the wavelength that is closest; however, the signal may vary significantly.

---

5 Instruments (for chemiluminescent)

Why don't my Relative Light Units (RLUs) compare to the kit insert?

- RLUs may vary significantly between luminometers because the industry has not standardized the measurement of light units. Values derived from the RLUs should be comparable.
- Use the suggested luminometer settings or equivalent settings if using a different luminometer. Differing these settings may cause a difference in signal.
- Read within the suggested reading time window.
- Chemiluminescent assays are kinetic and are not stopped. The signal will vary within the reading time window.

I followed the protocol but there is no signal, what may have happened?

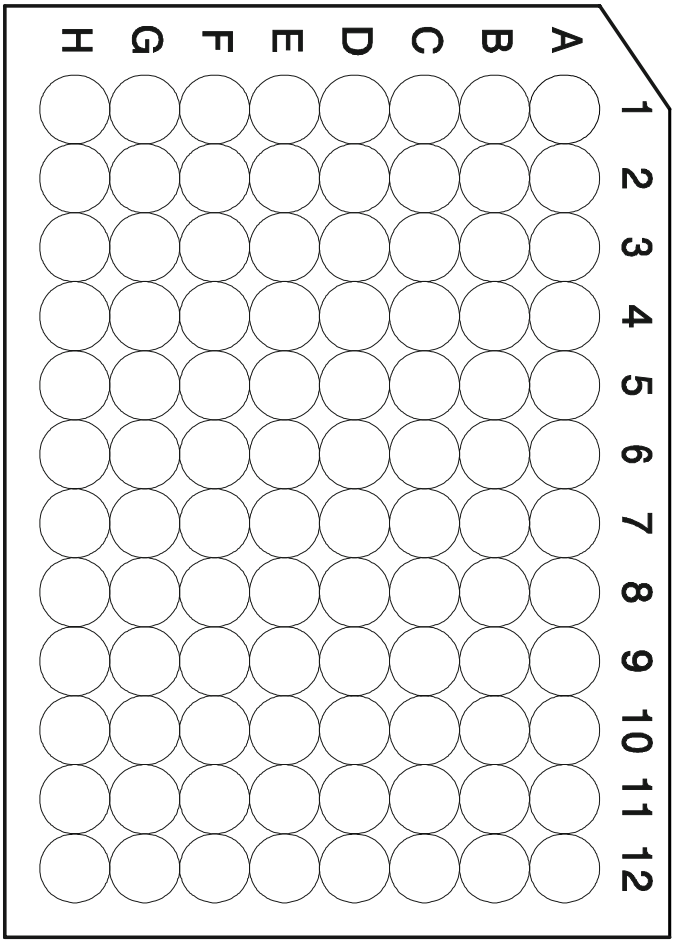
- Did you stop the plate? For colorimetric assays using HRP Substrate, the addition of stop solution changes the substrate color. The reading wavelength recommended is optimal for this color. Refer to the kit insert.
- Mix the reagents in the well by gently tapping the side of the plate. When Stop Solution is added, the color will change from blue to yellow (if it is an HRP Substrate). The substrate may turn to green if the reagents aren't mixed in the wells.
- The reagents may have been added in the wrong order.
- Don't wash the plate after the addition of substrate solution.
- Ensure that standard is added when performing serial dilutions for the standard curve.
- If the substrate system uses two components, the components need to be mixed. Refer to kit insert for preparation instructions.
- Ensure that the plate reader was set to the correct specifications. Refer to the kit insert.
- Ensure reagents, standards, and samples were prepared correctly and added to the wells according to the kit insert.
- For High Sensitivity (HS) kits, ensure that the appropriate diluents were used to reconstitute the Substrate and Amplifier. Refer to the kit insert.

What may cause high backgrounds (NSBs)?

- Altering incubation times or temperatures.
- Inadequate washing. Refer to Washing Techniques on page 4.
- Contamination of components.
- Contamination of plate during incubation.
- Reusing the plate sealers, reservoirs, or pipette tips may cause contamination.

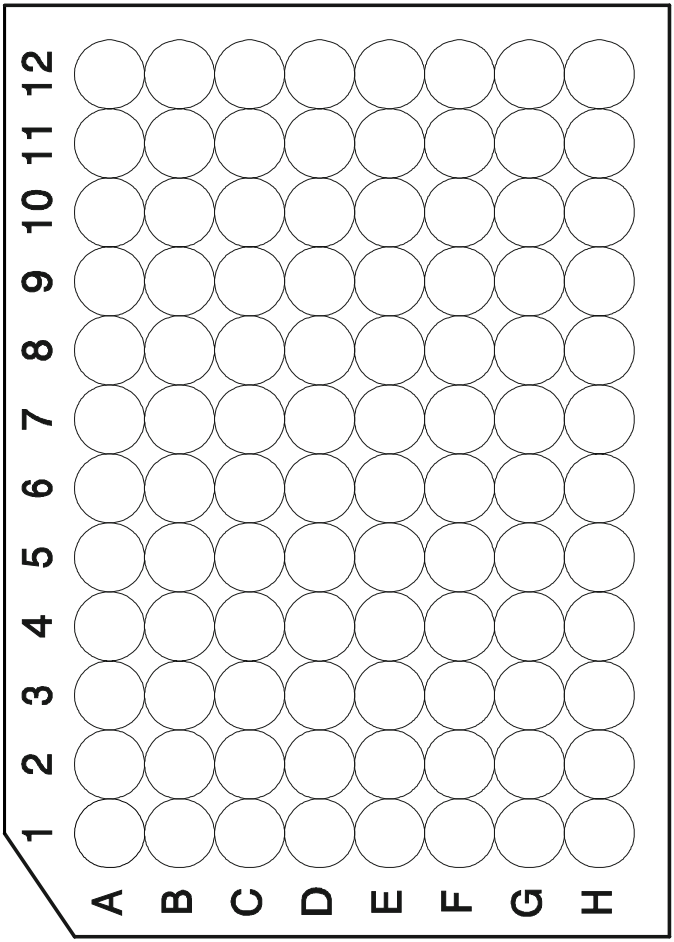
Notes

Plate Layout



This plate may be used as a record of standards and samples run in an assay.

Plate Layout



This plate may be used as a record of standards and samples run in an assay.