

TROUBLESHOOTING GUIDE

Poor Precision

Incomplete washing of wells

Ensure that wash apparatus is working correctly. Do not reduce wash volume, the number of wash steps or skip soak times.

Contamination

Saliva or skin may contain analyte. Wear mask or gloves.

Inadequate aspiration of wells

Wells should appear empty after aspiration. Blot plate on a clean paper towel after the last wash. Do not allow wells to overdry.

Inadequate mixing of reagents in the wells

Ensure adequate mixing of reagents in the wells.

Unequal volumes added to the wells

Check pipette function, recalibrate if necessary. Ensure that the pipette tips are securely attached. Use consistent pipetting technique.

Pipetting error

Repeat assay. Pipette standards and samples into the side of the wells to avoid splashing. Always run standards and samples in duplicate.

Reused pipette tips or reagent reservoirs

Change pipette tips between each standard, sample or reagent. Use separate reservoirs for each reagent.

Reused plate sealer

Use a new plate sealer for each incubation period as recommended in the kit insert.

Poor Standard Curve

Improper standard curve preparation

Ensure that the appropriate Calibrator Diluent is used. Reconstitute with the volume recommended in the kit insert. Avoid foaming when mixing or reconstituting and allow the standard to sit for the specified time before use. Ensure accurate completion of the dilution series.

Incomplete washing of wells

Ensure that wash apparatus is working correctly. Do not reduce the number of wash steps or skip soak times.

Inadequate aspiration of wells

Wells should appear empty after aspiration. Blot plate on a clean paper towel after the last wash. Do not allow wells to overdry.

Unequal volumes added to the wells

Check pipette function, recalibrate if necessary. Ensure that the pipette tips are securely attached. Use consistent pipetting technique.

Substrates prepared too early

Prepare substrate(s) within the time recommended in the kit insert.

Read beyond suggested reading window

Read within the time recommended in the kit insert.

Improper reduction method

Use the data reduction method recommended in the kit insert. Other reduction methods may give a less precise fit of the standard curve.

Pipetting error

Repeat the assay. Always run standards and samples in duplicate.

Inadequate Signal Development

Incorrect preparation of substrate

Ensure that the correct volumes of substrate reagents are used and are mixed properly. If the substrate is a tablet or is lyophilized, dissolve or reconstitute completely with the appropriate amount of diluent. Mix thoroughly and use within the time indicated.

Inadequate volume of substrate added to the wells

Check pipette function, recalibrate if necessary. Ensure that the pipette tips are securely attached. Use consistent pipetting technique.

Incorrect incubation times or temperatures

Adhere to the recommended incubation times and temperatures. Avoid incubating plates in areas where environmental conditions vary (e.g., under a vent or on a window sill). Time each plate individually to avoid over- or under-incubation.

Conjugate or substrate reagent failure

Check by mixing equal volumes of conjugate and substrate solution (for chemiluminescent assays add 10 μ L conjugate + 190 μ L substrate). Color or light should develop immediately. Store substrates in dark prior to use.

Improper instrument settings

Ensure that the correct wavelength filters are used on the microplate reader. Ensure that the correct settings are used with the luminometer. Refer to kit insert.

Read beyond suggested reading window

Read within the time recommended in the kit insert.

No stop solution added

Follow the assay protocol in the kit insert.

Common conversion factors used with ELISAs:

$$1\text{ L} = 1000\text{ mL} = 1 \times 10^6\ \mu\text{L}$$

$$1\text{ g} = 1000\text{ mg} = 1 \times 10^6\ \mu\text{g} = 1 \times 10^9\ \text{ng} = 1 \times 10^{12}\ \text{pg} = 1 \times 10^{15}\ \text{fg}$$

$$1\ \text{mol} = 1000\ \text{mmol} = 1 \times 10^6\ \mu\text{mol} = 1 \times 10^9\ \text{nmol} = 1 \times 10^{12}\ \text{pmol} = 1 \times 10^{15}\ \text{fmol} = 1 \times 10^{18}\ \text{attomol}$$

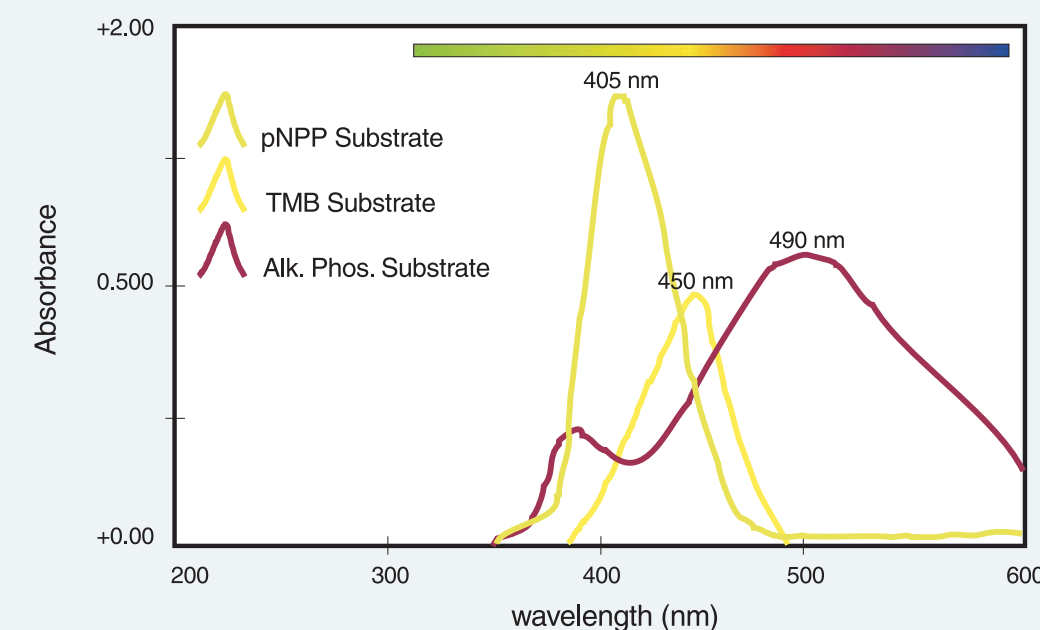
To convert between moles and grams, use the appropriate equation below:

$$\text{moles} = \frac{\text{grams}}{\text{MW}} \quad \text{or} \quad \text{grams} = \text{moles} \times \text{MW}$$

(MW: molecular weight in g/mol)

Equations for converting values into units/mL are provided in the individual product inserts.

Absorbance spectrum* showing the optimal wavelength for the substrates utilized in the R&D Systems colorimetric ELISAs.



**Colors are an approximate representation of the spectrum.*

Data Reduction

Improper data reduction method

Use the data reduction method recommended in the kit insert. Other reduction methods may give a less precise fit of the standard curve. If computer software is not available, plot the standard curve using log/log paper and apply regression analysis to the log transformation.

Standard curve not assayed

A separate standard curve must be run with each assay. The standard curve provided in the kit insert is for demonstration only and cannot be used to calculate results.

Edge Effect

Uneven temperatures around work surface

Avoid incubating plates in areas where environmental conditions vary (e.g., under a vent or window sill).

Inadequate sealing of plate cover, leading to evaporation

Ensure that the plate cover is adhered properly by firmly pressing the sealer along all four edges of the plate.

Drift

Interrupted assay set-up

Assay set-up should be continuous. Have all standards/samples prepared appropriately before commencement of the assay. Standards and samples should be added to the plate within 20 minutes unless indicated otherwise in the kit insert.

Incubation times and temperatures

Adhere to the recommended incubation times and temperatures. Avoid incubating plates in areas where environmental conditions vary (e.g., under a vent or window sill). Time each plate individually to avoid over- or under-incubation.

Reagents not at room temperature

Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the kit insert.

Incorrect luminometer settings

For chemiluminescent assays, read at 1.0 second/well. Reading at ≥ 2.0 seconds/well will produce a drift because of the time elapsed from the first wells read to the last wells read.

Sample Values

Improper collection or storage

Use the collection method recommended in the kit insert. If not assaying samples immediately, refer to kit insert for storage instructions.

Improper sample preparation

Sample preparation methods have been tested for optimal performance. Adhere to the recommended sample preparation instructions. Ensure that the correct Calibrator Diluent is used.

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