

TROUBLESHOOTING GUIDE FOR ENZYMATIC ASSAY KITS

1. Assay not working

Use of ice-cold assay buffer

Assay buffer must be at room temperature to work optimally.

Omission of a step in the protocol

Check and follow the datasheet precisely.

Plate read at incorrect wavelength

Check the wavelength recommended in the datasheet and the filter settings of the instrument.

Use of an unsuitable microtitre plate.

Fluorescence: Black plates (clear bottoms).

Luminescence: White plates.

Colorimetry: Clear plates.

Check datasheet whether to use flat or U-shaped wells.

2. Samples with inconsistent readings

Use of an incompatible sample type

Refer to the datasheet for details about suitable and incompatible samples.

Samples prepared in a non recommended buffer

Use the assay buffer provided in the kit or refer to the datasheet for instructions.

Samples were not deproteinized (if indicated in datasheet)

Use the 10 kDa spin cut-off filter or PCA precipitation as indicated in the instructions.

[View details](#) of our 10 kDa spin column.

[View details](#) of our Deproteinizing Sample Preparation Kit.

Cell/ tissue samples were not completely homogenized

Use a Dounce homogenizer (increase the number of strokes); observe for lysis under microscope to ensure sample is homogenized.

Samples used after multiple freeze-thaw cycles

Aliquot and freeze samples in small aliquots if they are intended for use in several experiments to avoid freeze – thaw cycles, which can damage the samples.

Presence of interfering substance in the sample

Some substances can interfere with assays and should be avoided in samples preparations. Examples of these are listed below. Please see individual kit datasheets for more specific information on substances that may affect the results from the kit you are using. Troubleshoot if needed and also consider deproteinizing samples. EDTA (>0.5 mM), Ascorbic acid (>0.2%), SDS (>0.2%), Sodium Azide (>0.2%), NP-40 and Tween-20 (> 1%) interferes with assays and should be avoided in samples preparations. Troubleshoot if needed.

Use of old or inappropriately stored samples

Use fresh samples or store at correct temperatures until use.

3. Lower/ Higher readings in Samples and Standards

Improperly thawed components.

Thaw all components completely and mix gently before use.

Use of expired kit or improperly stored reagents

Always check the expiration date and store the components appropriately as directed on the datasheet.

Allowing the reagents to sit for extended times on ice

Always thaw and prepare fresh reaction mix immediately before use.

Incorrect incubation times or temperatures

Refer to the datasheet and verify the correct incubation times and temperatures.

Incorrect amount used

Use calibrated pipettes and aliquot correctly.

4. Readings do not follow a linear pattern for Standard curve

Use of partially thawed components

Thaw and resuspend all components before preparing the reaction mix. This will ensure the reagents are in a homogenous solution.

Pipetting errors in the standard

Avoid pipetting small volumes.

Pipetting errors in the reaction mix

Prepare a master reaction mix whenever possible.

Air bubbles formed in well

Pipette gently against the wall of the tubes to eliminate air bubbles.

Standard stock is at an incorrect concentration

Always refer to the dilutions in the datasheet.

Errors in standard curve calculations

Refer to the datasheet and check the calculations.

Substituting reagents from older kits/ lots

Use fresh components from the same kit in order to provide standard results.

5. Unexpected results

Measured at incorrect wavelength

Check the equipment and the filter setting.

Samples contains interfering substances

Troubleshoot if it interferes with the kit.

Use of incompatible sample type

Refer to the datasheet to check if the sample is compatible with the kit or whether optimization is needed.

Sample readings above/below the linear range

Concentrate/ Dilute sample to ensure it provides results within the linear range. When calculating the results this dilution or concentration can be taken into account.