Frequently Asked Questions for processing the Nu.Q® ELISA Assays

Is it mandatory to centrifuge the samples 2 min at 14000g and to transfer the supernatant into a new tube?

Human samples: Yes. It is mandatory to centrifuge the K2-EDTA plasma samples 2 min at 14000g as well as to transfer the supernatant in a clean tube without disturbing the pellet. These two steps have been demonstrated as having a huge impact on the assay reproducibility and precision.

Dog samples: No. It is not recommended to centrifuge dog samples.

How should plasma samples be stored?

Human samples: It is recommended to store the K2-EDTA plasma samples at -80°C. We recommend to aliquot your samples in small volumes (~100µL) to avoid freeze thaw cycles. These aliquots will be one use only. We do not recommend a storage at -20°C.

Dog samples: K2-EDTA or K3-EDTA plasma samples cannot be stored at -80°C. Fresh samples must be used. Blood samples must be processed within 1 hour.

How should human plasma samples be defrost?

Human K2-EDTA plasma samples should be left 30 minutes to warm to room temperature (15-25 °C) prior to assay without the use of extra heating.

Do I need to wash the plates before use?

Yes. It is mandatory to wash the plates before use to remove the excess of blocking solution.

Can I use an automated washer?

The washing steps were not validated with an automated washer, only manually.

However, the wash solution in the Veterinary kit is supplied in a sufficient amount to be used with an automated washer. Use of automated microplate washer may also provide acceptable performance provided that it is first evaluated and validated by the laboratory to ensure equivalence and valid sample results.

Are the standard curve and kit controls stable at -20°C?

No, standards and kit controls are not stable -20°C.

After reconstitution, the standard curve and kit controls must be aliquoted and stored at -80°C. The aliquots are single use only (No freeze thaw cycle is allowed).

Can reagents from different batches be used?

No. Each batch of kit is optimized and validated to reach the expected performance described in the instructions for use. We recommend not to mix reagents from different lots.

Is it possible to avoid using a shaker?

It is mandatory to use an orbital shaker and to shake at 700rpm.

Can the protocol of the assay be modified?

The protocol of the assay is optimised to provide accurate results. Any modification or adaptation of the protocol will impact the accuracy of the assay.

Can timing of incubation steps of the assay be modified?

It is important to maintain incubation time in each well constant for reproducible results. Pipetting time for all samples should not extend beyond 15 minutes to avoid assay drift. If more than 15 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the standard curve in each plate.

Do we have to run the standard curve, kit controls and samples in duplicate in each run?

Yes, these values are used to calculate the concentration of the samples. They reflect day to day, operator to operator variations. Running the standard curve and kit controls monitor assay precision and accuracy.

What is the storage temperature of the kit?

Before use, store the reagents at 2-8°C. After opening, the reagents are to be stored at 2-8°C except the standard curve and kit controls. They must be stored at -80°C aliquoted after reconstitution.

Can serum or other sample types be used in this kit?

Human samples: Only K2-EDTA plasma samples have been validated.

Dog samples: Only K2-EDTA and K3-EDTA plasma samples have been validated.

Other samples type may be used at the risk or the responsibility of the customer.

What are the possible reasons for high background?

Improper washing: Check if the washing steps have been performed correctly.
Contaminated substrate: Make sure there is no contamination of the substrate.
Substrate exposed to light: Exposure to light may result in a blue colour of the substrate. Keep solutions in the dark until ready to dispense into the plate.

What are the possible reasons for high CV%?

Improper pipetting: Check correct calibration of your pipettes and appropriate tips used
Improper washing: Check if the washing steps have been performed correctly.
Sample/standard inhomogeneity: Make sure that the samples were correctly centrifuged and transferred in a new tube without touching the pellet. Make also sure that the standards and samples were well vortexed before use.
Contaminated substrate: Make sure there is no contamination of the substrate.
Wrong mixing: please check the plates were mixed with orbital shaking at 700 rpm.

Which method or software can be used to analyse the data?

It's recommended to use a 4-parameter logistic function with a weighing factor $1/Y^2$ and if possible, use prism software (GraphPad).

What material is required but not provided?

For correct performance of the assay, the following material should be used. Please check their availability before starting the assay:

- Purified water.
- Adjustable or preset single-channel pipettes able to dispense 20 µL.
- Multi-channel pipettes able to dispense 50 µL to 200 µL.
- Disposable pipette tips.
- Disposable reagent reservoirs.
- Vortex mixer.
- Volumetric cylinders.
- Container for biohazardous waste.
- Microplate incubator with orbital shaking facility (shaker at 700 rpm).

- Microplate reader equipped with 450 nm filter and able to read optical density between 0 and 4.0.
- Absorbent paper.
- Light-tight plate cover.

REMARK: For best assay efficiency, we recommend the use of qualified equipment:
- calibrated pipettes and use of adequate tips according to supplier information
- calibrated shaker
- calibrated plate reader.

What should I do if the mean value of the controls are out of specifications?

The mean value of the different controls must be within the specifications described in the table below:

Control	Acceptable range
Standard curve A	OD value < 0.20
Kit Control 1	See range in Certificate of Analysis
Kit Control 2	See range in Certificate of Analysis

Any value out-of-range for these controls invalidates the assay which must be performed again. Each lab should assay controls at different levels of H3.1 nucleosome for monitoring assay performances. These controls should be treated as unknowns and values determined in every test procedure performed.

Can I dilute specimen?

The test must be performed on **undiluted** plasma samples. Samples with concentrations above the reportable range cannot be quantified with accuracy. A concentration can be estimated by extrapolating values using a 4-parameter logistic function curve fitting.

For more information on correct handling of the Nu.Q ELISA product, please check video on https://vimeo.com/667215896?&login=true# =

Any questions please email info@diapharma.com