

IFU – Nu.Q® H3R8Cit ELISA Assay - RUO
For Research Use Only. Not for use in diagnostic procedures.

Important Note:

This is a Research Use Only (RUO) product under development by Volition and has not been produced within any quality management system. This RUO product is **not** intended for clinical diagnosis, patient management, or any an investigation that is not exempt from 21 CFR part 812.

1. REAGENTS, MATERIALS AND INSTRUMENTATION

1.1. Reagents and materials supplied in the kit

- a) Standard/Calibrators (6 vials, 250µL each)
- b) Kit Controls 1&2 (2 levels, 250µL)
- c) Conjugate (1 vial, 13 mL) Anti-nucleosome antibody conjugated with horseradish peroxidase (HRP)
- d) Coated Microplate (1 breakable microplate) Anti-H3R8 Citrulline antibody adsorbed on wells
- e) Assay buffer (1 vial 13mL)
- f) TMB Substrate (1 vial, 13 mL)
- g) Stop Solution (1 vial, 13 mL) Chlorhydric acid 1M (avoid any skin contact)
- h) 10X Conc. Wash Solution (1 vial, 30 mL) Phosphate buffer 40mM, proclin 0.0075%, Tween 20 0.5%
- i) Plate foil (3X).

1.2. Materials required but not provided

- 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 a qualified calibrated pipettes and the use of corresponding tips according to supplier information, qualified calibrated shaker, and qualified calibrated reader.

2. STORAGE CONDITIONS & SHELF-LIFE

The kit must be stored at 2-8 °C, apart from standard and kit controls stored at -80°C.

At this temperature, the unopened reagents contained in the Nu.Q® H3R8cit test may be used up to the expiry date printed on the labels. Do not use reagents beyond this date.

3. WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents contain small amounts of Proclin 300 as preservative. Avoid contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted chlorhydric acid solution. Chlorhydric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.

4. PROCEDURE

4.1 PREPARATION OF THE REAGENTS

- **Standard curve and kit controls**

The Standard curve and the Kit Controls 1 and 2 are supplied frozen and must be stored at -80°C.

They must be transferred to room temperature for 30min to defrost before use.

Mix gently with a rotating mixer before loading on plate.

Once defrosted, aliquot the remaining volume of each Standards and Kit Controls and store at -80°C

Once an aliquot is defrosted and used again, the remaining volume has to be discarded. Avoid freeze/thaw cycles of the aliquoted Standards and Kit Controls.

NOTE:

The concentration of the standard curve and the range of the kit controls is lot specific and is provided in the Certificate of Analysis.

- **Wash buffer**

Dilute 10-fold. For example, 50 mL of 10X concentrated Wash buffer may be added to 450 mL deionized water to a final volume of 500 mL.

The diluted wash buffer may be stored at 2-8 °C for up to 14 days.

Note: It is possible to observe the presence of crystals in the concentrated wash solution. In this case, mix at room temperature until complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

- **Coated microplate**

Open the coated microplate only when it is at room temperature. Unused coated microwell strips should be sealed securely in the foil pouch containing desiccant and stored at 2-8 °C.

Once opened, the microplate is stable up to 14 days at 2-8 °C.

- **Specimen**

The determination of H3R8cit nucleosome concentration must be performed on **frozen** K2EDTA Plasma samples (-80°C).

4.2 TEST PROCEDURE

- **General remarks**

- Follow the instructions carefully. Once started, all test steps should be completed without interruption.
- Use a new disposable plastic pipette tip for each control or sample to avoid cross contamination.
- Each reagent reservoir must be dedicated to a unique reagent. Never reuse or mix reservoirs that have contained different solutions.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred back into the original vials.
- Once used, store immediately the reagents at 2-8 °C. Avoid long exposure to room temperature.

- Avoid exposure of reagent TMB substrate to direct sunlight, metals or oxidants. Do not freeze the solution.

Always use all reagents from a single lot of the Nu.Q® H3R8cit Test.
The reliability of results across different lots has not been validated.

- **Assay procedure**

- Assay preparation

Each assay must include a complete standard curve (Standard 1 to 6) and 2 kit controls allowing proper quantification of samples.

1. Carefully establish the plate design. All standard curve points, kit controls and samples should be run at least in duplicate.
2. Prepare the Standard curve, Kit controls and diluted Wash buffer.
3. Allow the reagents to warm up for 30min to room temperature (15-25 °C) and homogenize each of them before use.
4. Vortex all plasma samples, standard curve points and kit controls to homogenize them.

**Before performing the measurement,
the plasma samples must be centrifuged 2 min at 14000 g.
After separation, transfer the supernatant into a clean tube.**

- Manual procedure

1. First allow all reagents to reach room temperature (15-25 °C) for at least 30 minutes before use.
2. **Before use, wash the wells 3 times with 200 µL of Wash Buffer.** Remove each time the excess solution by tapping the inverted plate on an absorbent paper towel (e.g. paper tissue or towel).
3. Dispense 80 µL of Assay Buffer into each well.
4. Dispense 20 µL of each sample, standard curve point or kit control into the appropriate wells according to your plate design. Use a new disposable pipette tip for each sample.
5. Cover the microplate with a sealing film. Press firmly to ensure a tight seal.
6. Incubate the microplate at 15 °C to 25 °C on a microplate incubator with orbital shaking at approximately 700 rpm for 2.5 hours.
7. Remove the adhesive film. Empty the contents of all wells by decanting and tapping the microplate on an absorbent material (e.g. paper tissue or towel) and wash the wells 3 times with 200 µL diluted Wash Buffer. Following each

- washing step, remove the Wash buffer from the plate by decanting and tapping the plate on an absorbent material.
8. Add 100 µL of HRP labelled detection antibody into each well. Cover the plate with a sealing film and incubate at 15 °C to 25 °C for 90 minutes on a microplate incubator with orbital shaking at approximately 700 rpm.
 9. Repeat the washing procedure described in step 7.
 10. Add 100 µL of TMB substrate solution into each well. Allow the color reaction to develop in the dark for 20 minutes at 15 °C to 25 °C with gentle shaking on a microplate incubator with orbital shaking at approximately 700 rpm in the dark (cover the plate with a light-tight cover, do not use adhesive film during this incubation)
 11. After 20 min, stop the colorimetric reaction by adding 100 µL of Stop solution to each well. Use the same sequence and rate of dispensing as for the substrate solution. Mix by placing the plate for 1 min on the microplate incubator with orbital shaking at 700 rpm.
 12. Within the next 5 minutes, determine the optical density (OD) of the wells at 450 nm using a microplate reader.

5. QUALITY CONTROL

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.

6. CALCULATION AND INTERPRETATION OF RESULTS

6.1 Calculation of the mean OD value and coefficient of variation for the samples

Before sample analysis, the mean absorbance and coefficient of variation of each sample must be calculated as follow:

$$\text{Mean OD (} \overline{OD} \text{)} = \frac{OD \text{ duplicate 1 (OD 1) + OD duplicate 2 (OD 2)}}{2}$$

$$\text{Standard Deviation (Sd)} = \sqrt{\frac{(\text{OD } 1 - \overline{\text{OD}})^2 + (\text{OD } 2 - \overline{\text{OD}})^2}{2}}$$

$$\text{Coefficient of Variation (\%CV)} = \frac{\text{Sd}}{\overline{\text{OD}}} \times 100$$

Any sample result with a coefficient of variation above 20 % must not be considered as valid and must be re-assayed.

Note: Use of a computer assisted method (e.g. *Prism* (GraphPad)) is recommended.

6.2 Interpretation of the results

Interpretation of the results must be performed by interpolating the mean OD value of each tested sample against the standard curve and expressing them in ng/mL. To do so, the construction of the standard curve using of a **4-parameter logistic function curve fitting with a weighing factor 1/Y² is required**.

Note: Use of a computer assisted method (e.g. *Prism* (GraphPad)) is recommended. If the laboratory has no license for Prism, a trial version for 30 days can be downloaded from Graph pad website (<https://www.graphpad.com/demos>).

7. PRECAUTIONS & LIMITATIONS OF USE

The reliability of the results depends on correct implementation of good safety and hygiene principles including:

- Do not use reagents beyond the expiry date on the labels.
- Do not use if you see damaged plate packaging.
- Please adhere strictly to the sequence of pipetting steps and reagent preparation steps provided in this protocol.
- Replace caps on reagents immediately after use. Do not switch caps.
- Do not allow the microplate to dry between the end of the washing operation and dispensing of the next reagent.
- Use a new pipette tip to dispense each patient specimen.
- Never use the same reservoir to distribute the HRP and substrate solution.
- Well washing is a critical step: adhere to the recommended number of washing cycles and make sure that all wells are filled and then completely emptied. Incorrect washing may lead to inaccurate results
- Do not change the assay procedure. All indicated volumes must be performed according to the protocol.

- Allow all kit components and specimens (Plasma K2EDTA) to reach room temperature (15-25°C) at least for 30min and mix well prior to use.
- Plasma K2 EDTA samples should be left to warm to room temperature (15-25 °C) prior to assay without the use of extra heating.
- Incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of 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 dose response curve in each plate.
- Maximum precision is required for dispensation of the reagents.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Do not mix components from different kits lots.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested. To improve the performance of the kit on ELISA automatic systems, it is recommended to increase the number of washes.

Other recommendations :

- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Quality control should be performed every time the assay is run.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.

Please consider:

- RUO Reagents are under development to determine production methods, purification levels, packaging needs, shelf life, storage conditions, etc.
- Volition has not completed stability studies for this RUO assay so the Shelf Life shall be derived from the dates of the subcomponents.
- Variability between batches is possible.
- Volition's ability to support the Customer's use of the RUO kit will be limited. Please work with your Commercial representative for questions.