# GENLISA™ Human Aquaporin 4 Antibody, AQP-4 Ab ELISA

**REF** : KBH4979

Ver 2.1

**RUO**

Enzyme Immunoassay for the Qualitative Determination of Human Aquaporin 4 Antibody, AQP-4 Ab in human serum, plasma and other biological samples.

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|---|---|---|---|
| **RUO** | **For Research Use Only** | **REF** | **Catalog Number** |
| | **Store At** | **LOT** | **Batch Code** |
| | **Manufactured By** | | **Biological Risk** |
| | **Expiry Date** | | **Consult Operating Instructions** |

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**REF KBH4979** **Σ 96 tests**

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**Introduction:**

The GENLISA™ ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody.

**Intended Use:**

The GENLISA™ Human Aquaporin 4 Antibody, AQP-4 Ab ELISA kit is used as an analytical tool for qualitative determination of Human Aquaporin 4 Antibody, AQP-4 Ab in human serum, plasma and other biological samples.

**Principle:**

The method employs the sandwich enzyme immunoassay technique. Human Aquaporin 4 Antigen is pre-coated onto microwells. Samples and controls are pipetted into microwells and Aquaporin 4 Antibody, AQP-4 Ab present in the sample are bound by the Antigen. Then, HRP Conjugate is pipetted and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Aquaporin 4 Antibody, AQP-4 Ab in the sample. The color development is stopped by addition of stop solution. Absorbance is measured at 450 nm.

**Materials Provided:**

1. Human Aquaporin 4 Antigen Microtitre coated plate – 1 x 96 well
2. Positive Control - 0.5 ml
3. Negative Control - 0.5 ml
4. HRP Conjugate - 6 ml
5. Sample Diluent - 6 ml
6. 20X Wash Buffer - 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction manual

**Materials to be provided by the End-User:**

1. 37ºC incubator
2. Standard microplate reader.
3. Precision pipettes and Disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution
6. Absorbent paper.

**Storage Information:**

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

**Sample Preparation and Storage:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. **Serum-** Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. **Plasma-** Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
6. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

***Note:*** *Grossly hemolyzed samples are not suitable for use in this assay.*

**Reagent Preparation (all reagents should be diluted immediately prior to use):**

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X)**; dilute **25ml** of **20X Wash Buffer** in **475 ml** of **DI water**.

**Procedural Notes:**

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human Aquaporin 4 Antibody, AQP-4 Ab. High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Aquaporin 4 Antibody, AQP-4 Ab present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent.
3. Human Aquaporin 4 Antibody, AQP-4 Ab concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide ($NaN_3$), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Aquaporin 4 Antibody, AQP-4 Ab.
5. It is recommended that all Controls and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Controls and Samples.

**Assay Procedure:**

1. Bring all reagents to room temperature prior to use. It is strongly recommended that Samples should be run in duplicates.

2. **Add 50 ul Negative control and 50 ul positive control to respective control wells**;

3. Add Sample diluent **40 ul** to testing sample well, then add testing sample **10 ul** (sample final dilute degree is 5 times)

4. Incubate at 37°C for 30 minutes.

5. Aspirate and wash plate 5 times with 300 ul Wash Buffer (1x) and blot residual buffer by firmly tapping plate upside down on an absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

6. Add **50 ul of HRP Conjugate** to each well (Do not add into blank control well)

7. Incubate at 37°C for 30 minutes.

8. Aspirate and wash plate **5 times** with **300 ul Wash Buffer (1x)** and blot residual buffer by firmly tapping plate upside down on an absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

9. Pipette **100 ul of TMB Substrate to each well.**

10. Incubate at 37°C for 10 minutes.

11. Add **100 ul of Stop Solution** into all wells(Do not add into blank control well)

12. Calibrate the plate reader with blank control well and read the plate using microwell plate reader at 450 nm

**Calculation of Results:**

The Cut Off = Mean of the Negative Control + 0.15

**Validity of the test:**

Mean of the Positive Control ≥ 1.00;
Mean of the Negative Control ≤ 0.15

**Interpretation of Results**

Negative determinant: if the sample OD value < Cut Off , the Human Hepatitis-B Virus Core IgM is Negative;
Positive determinant: if the sample OD value ≥ Cut Off , the Human Hepatitis-B Virus Core IgM is Positive.

**Safety Precautions:**

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from Human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed

- Do not smoke, eat or drink while handling kit material

- Always use protective gloves

- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

**Typical Example of a Work List**

| Well # | Contents | Absorbance at 450nm | Mean Absorbance | Interpolated Concentration |
|---|---|---|---|---|
| 1A<br>2A | Positive Control<br>Positive Control | | | |
| 1B<br>2B | Negative Control<br>Negative Control | | | |
| 1C<br>2C | Sample | | | |
| 1D<br>2D | Sample | | | |
| 1E<br>2E | Sample | | | |
| 1F<br>2F | Sample | | | |
| 1G<br>2G | Sample | | | |
| 1H<br>2H | Sample | | | |
| 3A<br>4A | Sample | | | |
| 3B<br>4B | Sample | | | |

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## SYMBOLS KEY

| | |
|---|---|
| MTP | Coated Microtiter Plate (8 x 12 wells) |
| POS CNTRL | Positive Control |
| NEG CNTRL | Negative Control |
| HRP CONJ | HRP- Conjugate |
| SAMP DIL | Sample Diluent |
| 20X WASH BUF | (20X) Wash Buffer |
| SUB TMB | TMB Substrate |
| SOLN STOP | Stop Solution |
|  | Consult Instructions for Use |
| REF | Catalog Number |
|  | Expiration Date |
|  | Storage Temperature |