Human Quinolinic Acid (QA) ELISA Kit
96T

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FIVEphoton Biochemicals

For research use only.
Not for diagnostics.

Part No. hQA-ELISA1 (96T)

FIVEphoton Biochemicals
4907 Morena Blvd, Ste 1403
San Diego, CA 92117
Tel: 858 395-4026
Fax: 858 345-5291
Website: www.fivephoton.com
customersupport@fivephoton.com

Store: 4^{o}C. Expiration: 1 year.
Safety: Stop solution contains acid. Avoid contact and inhalation. Wear eye protection.

Quinolinic Acid (QA) ELISA Kit - Human Specificity

Part No.

Standard Concentration: 64 ng/ml

Assay Range: 0.2 ng/ml to 60 ng/ml

Sensitivity: 0.187 ng/ml

For research only. Not for diagnostic applications.

Storage: 2-8^{o}C, 6 months

Safety: Stop solution contains acid. Avoid eye and skin contact

Overview: Please familiarize yourself with this protocol prior to performing the assay. It is recommended that the researcher overviews the literature to identify detailed methods for sample preparation; the sample isolation methods discussed in this protocol serve only as a general guide. Blanks, standards and samples should be measured at least in duplicate to account for pipetting error.

This kit measures quinolinic acid (QA) in sandwich ELISA format. The ELISA plate is provided pre-coated with an anti-QA monoclonal antibody. The experimenter mixes the samples with a provided polyclonal anti-QA antibody labeled with biotin and with the provided streptavidin-HRP solution. The sample-antibody-biotin-streptavidin-HRP mixture is then added to the ELISA plate, which is incubated for 60 min at 37^{o}C. Unbound biotin-linked antibody is washed away and color reagents are added that change the color of the solution to blue. The blue solution then turns yellow after addition of stop solution. The absorbance of the resulting yellow solution at 450nm corresponds to the concentration of QA in the samples.

Sample Preparation: The below is provided only as a general guide. Prior to performing the assay, the researcher should identify sample preparation methods for their experiment.

1. Note that samples containing NaN_3 may inhibit Horse Radish Peroxidase (HRP) and the color reaction.
2. If possible, assay the sample immediately after isolation. Otherwise store samples at -80^{o}C without freeze-thawing cycles.
3. **Serum**: Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant carefully. If particulates are detected after storage, centrifuge again and collect the supernatant for assays. A significant dilution (for example, 200-1000X fold) of serum in a sample diluent solution (1% BSA in PBS pH 7.4 – made by experimenter, not provided with the kit) may be required to maintain assay specificity.
4. **Blood plasma**: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as the anti-coagulant. Add EDTA or sodium citrate and mix 10-20 minutes. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. If particulates are detected after storage, centrifuge again and collect the supernatant for assays. A significant dilution (for example, 200-1000X fold) of plasma in a sample diluent

solution (1% BSA in PBS pH 7.4 – made by experimenter, not provided with the kit) may be required to maintain assay specificity.

5. **Urine**: Collect by sterile tube. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. When particulates appear due to storage, centrifuge again and collect the supernatant to assay.

6. **Cell culture supernatant**: Collect in sterile tubes when examining secreted components. Centrifuge at 2000-3000 RPM for approximately 20 minutes to sediment cells. Collect the supernatants carefully.

7. **Intracellular and membrane bound components**. To assay intracellular components, use a non-denaturing cell lysis buffer to lyze membranes and solubilize cytoplasmic and membrane bound components. Add ice cold lysis buffer with protease inhibitors to cells. Keep the tube in ice for 30 min. Tap the tube several times during this period to mechanically shear membranes. Centrifuge cellular debris in a microcentrifuge at 18000 rpm for 15 min. Collect the supernatant to assay.

8. **Tissue sample**: Use a homogenization protocol in a non-denaturing buffer to release protein components. Add protease inhibitors and keep the solution ice cold during the homogenization steps. Centrifuge debris and assay the supernatant.

Do not use denaturing cell lysis buffers such as RIPA for protein extraction.

9. Samples can be aliquoted and stored at -80^{o}C for later use.

Table 1. Materials supplied. Store all materials at 4^{o}C. If particulates are observed in any of the reagents, centrifuge and use the clarified supernatant for the assay.

1	Standard : (64ng/ml)	0.5ml	7	Chromogen Solution A	6ml
2	Standard diluent (1%IgG-free BSA in PBS pH 7.4)	3 ml	8	Chromogen Solution B	6ml
3	Anti-QA monoclonal antibody coated ELISA plate (96T)	12 well× 8 strips	9	Stop Solution	6ml
4	Streptavidin-HRP	6 ml	10	Instruction Manual	1
5	30×wash solution	20 ml	11	Closure Membrane	2
6	Anti-QA polyclonal antibody labeled with biotin (detection antibody)	1ml	12	Sealed bags	1

Materials required but not supplied

1. Sample dilution buffer (1% IgG-free BSA in PBS pH 7.4)
2. 37^{o}C incubator
3. Standard absorbance plate reader
4. Precision pipettes and disposable pipette tips
5. De-ionized water
6. Disposable tubes for sample dilution
7. Absorbent paper

Important notes and preparation for the assay

1. Make a solution that is 1% IgG-free BSA in PBS pH 7.4 for dilution of samples (i.e. ”sample dilution solution”).
2. Equilibrate the ELISA plate strips and other reagents used in the assay to room temperature for at least 30 minutes. The plate strips should be kept in a zip lock bag to prevent drying of the wells. Store unused sections of the ELISA plate in a sealed bag at 4^{o}C.

3. Use new pipettes tips at each step to prevent contamination.
4. Do not use reagents from other ELISA kits.
5. Substrate B is light sensitive. Limit light exposure.
6. **Manual Wash Method:** Gently aspirate off the liquid in each well. Turn over the plate and pat-dry on absorbent paper. Add 100µl wash solution and let percolate in the wells for 3 minutes prior to aspirating. Repeat the wash steps 5 times with 30 second washes. Therefore 600ul wash solution is needed per well. An automatic washer can also be employed to wash the ELISA wells.
7. **Very Important**: Perform preliminary tests to identify the appropriate dilution of your samples to match the linear range of the ELISA kit. For example, remove a 8-well strip, prepare one well with standard at the most concentrated dilution and another well with standard at the most dilute concentration of the assay range listed on Table 2. Set one well as the bank well (see blank preparation below), and perform a dilution series of your representative samples at 10X diluted increments for the remaining five wells. Then perform the assay. Identify the dilution factor for your samples that provides a measurement at approximately mid-point of the assay range for your samples. Dilute your samples with the sample dilution solution (1% IgG-free BSA in PBS pH 7.4) prior to applying the samples to the ELISA plate.

Assay procedures

Blanks, standard and sample preparation: Prepare the blanks, standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA plate. Do not prepare samples in the ELISA plate.

Assay procedure

1. Standard dilutions. Use Table 2 as a guide for dilutions of standards in the "standard diluent" provided with the kit (Table 1). This dilution series is applicable for a standard that is 64ng/ml that is provided with the Human QA ELISA kit. Make dilutions in separate tubes or a separate multi-well dish, but NOT in the ELISA dish.

Table 2. Standard Dilutions

Standard Concentration	Standard Number	Dilution Instructions
32 ng/ml	7	120µl original standard + 120µl standard diluent solution, mix
16 ng/ml	6	120µl standard No.7 + 120µl standard diluent solution, mix
8 ng/ml	5	120µl standard No.6 + 120µl standard diluent solution, mix
4 ng/ml	4	120µl standard No.5 + 120µl standard diluent solution, mix
2 ng/ml	3	120µl standard No.4 + 120µl standard diluent solution, mix
1 ng/ml	2	120µl standard No 3 + 120µl standard diluent solution, mix
500 pg/ml	1	120µl standard No 2 + 120µl standard diluent solution, mix

2. Blank, standard and sample preparation: (Pre-mix solutions in a separate multiwell dish and transfer the solutions simultaneously to the ELISA dish. Do not pre-mix solutions in the ELISA dish).

a) Blank wells: Set up two blank wells. Reproduce the sample dilution solution and sample vehicle solution ratio for the blanks; designate this mixture as the "blank solution". For each blank well, prepare 40 µl of "blank

solution". Then add 10µl anti- QA-biotin detection antibody and 50µl Streptavidin-HRP solution. Mix gently; do not vortex.

b) Standard solution wells: Prepare 50µl of diluted standard and 50µl Streptavidin-HRP solution for each standard well (*detection antibody labeled with biotin has been added during manufacture in advance for the standards, therefore no detection antibody label with biotin is added to the standards*).

c) Sample well: For each well, prepare 10ul sample (that may have been previously diluted to meet the assay range) in 30µl sample diluent solution (1% BSA in PBS pH 7.4 – made by the experimenter), then add 10µl anti-QA-biotin detection antibody and 50µl Streptavidin-HRP solution. Mix gently; do not vortex. Accurately transfer 100µl of the premixed solutions to the ELISA dish. (You may opt to prepare a slight surplus volume of standard, sample and blank solutions (for example 105 µl) using these reagent ratios to assure 100µl of solution is accurately transferred to each well during the pipetting step). Seal the wells with the seal plate membrane and rotate the plate gently for 60 minutes at 37°C.

3. Preparation of wash solution: Dilute the 30X wash solution to 1X with dH_20. Prepare 600µl 1X wash solution per well.
4. Wash: Carefully remove the seal plate membrane or zip lock bag that protects the wells from drying: do not cross-contaminate liquid. Gently aspirate off the liquid in each well. Turn over the plate and pat-dry on absorbent paper. Add 100µl wash solution and let percolate in the wells for 3 minutes prior to aspirating. Repeat the wash steps 5 times with 30 second washes. Therefore 600ul wash solution is needed per well. An automatic washer can also be employed to wash the ELISA wells. Blot dry the plate but do not allow the wells to dry.
5. Color development: First add 50µl chromogen solution A to each well and then add 50µl chromogen solution B to each well. Shake gently to mix solutions A and B. Incubate protected from light for 10 minutes at 37°C.
6. Stop: Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow).
7. Read samples at 450nm within 10 min of adding the stop solution: Set the blank wells as zero, measure the absorbance (OD) of each well at 450nm.

Data Analysis

1. Compile a standard curve using the blank standard solutions and the corresponding OD values. You may wish to calculate a linear regression equation from the standard curve to determine the concentration of your samples. Take into account dilution factors in your calculation. Alternative methods of data analysis to calculate concentrations of your samples can also be employed.

Flow chart of the procedures

Separately prepare standards, blank and samples with detection antibody and HRP-streptavidin as required

Incubate with the ELISA plate for 1 hr at 37°C.

Wash the plate five times.

Add Chromogen solutions A and B. Incubate for 10 min at 37^{o}C for color development.

Add stop solution

Measure OD value at 450nm within 10 min

Fivephoton Biochemicals, 4907 Morena Blvd, Ste 1403, San Diego,CA 92117
'Tel: 800.462.4507, Fax: 858-345-5291