Version 02-05/24

User's Manual

Kynurenic Acid ELISA kit

REF **DEIASL169**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the quantitative measurement of KYNA in serum, plasma and other biological fluids.

General Description

Kynurenic acid (KYNA) is a downstream metabolite produced along the Kynurenine Pathway well-known to possess several neuromodulatory functions, as well as immuno-modulatory properties. Measurement of KYNA in biological samples has been presented as a possible biomarker in several diseases including neurological or psychiatric disorders.

Principles of Testing

Kynurenic Acid ELISA Kit is based on a competitive enzyme immunoassay technique. The microtiter well-plate in this kit has been pre-coated with an anti-KYNA antibody. Sample or standards are added to the wells along with a fixed quantity of biotinylated KYNA and incubated. The KYNA found in the sample or standards competes with the biotinylated KYNA for limited binding sites on the immobilized anti-KYNA antibody. Wells are washed and Avidin-HRP conjugate is added, incubated then washed. An enzymatic reaction is then produced through the addition of TMB substrate which is catalyzed by the immobilized HRP to generate a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration is measured by reading the absorbance at 450 nm which is quantitatively proportional to the amount of biotinylated KYNA captured in the well and inversely proportional to the amount of KYNA which was contained in the sample or standard.

Reagents And Materials Provided

1. Anti-KYNA Microplate: 96 Wells (12 x 8 Well strips). Store at -20°C for 6 months.
2. KYNA Lyophilized Standard: 2 x 1000 ng. Store at -20°C for 6 months.
3. KYNA-Biotin Complex (100x): 1 vial (Lyophilized). Store at -20°C for 6 months.
4. 100X Avidin-HRP Conjugate: 1 x 120 µL. Store at -20°C for 6 months.
5. Reconstitution Buffer: 1 x 300 µL. Store at -20°C for 6 months.
6. Biotin Complex Diluent: 1 x 12 mL. Store at 4°C for 6 months.
7. Conjugate Diluent: 1 x 12 mL. Store at 4°C for 6 months.
8. 30X Wash Buffer: 1 x 20 mL. Store at 4°C for 6 months.
9. Standard Diluent: 1 x 20 mL. Store at 4°C for 6 months.
10. TMB Substrate: 1 x 9 mL. Store at 4°C for 6 months.
11. Stop Solution: 1 x 6 mL. Store at 4°C for 6 months.

Materials Required But Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

Storage

Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for 6 months or until expiration date. Avoid any freeze/thaw cycles.

Specimen Collection And Preparation

• Serum - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at 1,000 x g. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• Plasma - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

• Other biological fluids - Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Sample Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.

• Dilute samples using Standard Diluent.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

• Optimal dilution must be determined by the user according to their specific samples

Reagent Preparation

Equilibrate all materials to room temperature prior to use and use immediately.

KYNA Assay Standards

1. Prepare the KYNA standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.
2. Reconstitute one vial of the provided 1000 ng Lyophilized KYNA Standard for each experiment. Prepare a stock 2000 ng/mL Standard by reconstituting one tube of Lyophilized KYNA Standard as follows:

(1) Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

(2) Add 500 µL of Standard Diluent to the vial.

(3) Seal the vial then mix gently and thoroughly.

(4) Leave the vial at ambient temperature for 15 minutes.

3. Prepare a set of serially diluted standards as follows:

(1) Label tubes with numbers 2 – 6.

(2) Use the undiluted 2000 ng/mL KYNA Standard from step 2 as the high standard point (Tube #1).

(3) Add 600 µL of Standard Diluent to Tube #'s 2 – 6.

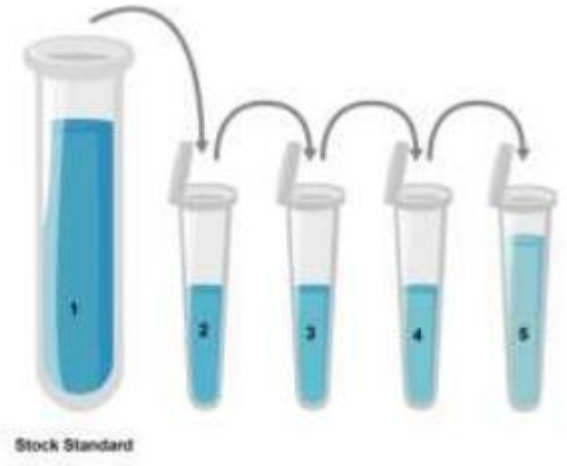
(4) Prepare Standard #2 by adding 300 µL of 2000 ng/mL KYNA (Tube #1) to Tube #2. Mix gently and thoroughly.

(5) Prepare Standard #3 by adding 300 µL of Standard #2 from Tube #2 to Tube #3. Mix gently and thoroughly.

(6) Prepare further serial dilutions through Tube #6. Reference the table below as a guide for serial dilution scheme.

(7) Tube #6 is a blank standard (only Standard Diluent), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Standard Diluent (µL)	Total Volume (µL)	Final Concentration
1	1,000 ng/mL Reconstituted Standard	NA	500	500	2,000 ng/mL
2	2,000 ng/mL	300	600	900	666.67 ng/mL
3	666.67 ng/mL	300	600	900	222.22 ng/mL
4	222.22 ng/mL	300	600	900	74.07 ng/mL
5	74.07 ng/mL	300	600	900	24.69 ng/mL
6	NA	0	600	600	0.0 (Blank)



1X KYNA-Biotin Complex

1. First, prepare the 1X KYNA-Biotin Complex by adding 150 µL of Reconstitution Buffer to the vial KYNA-Biotin Complex. Mix gently and thoroughly. Use immediately.
2. Dilute the 100X KYNA-Biotin Complex 1:100 with Complex Diluent to prepare a 1X KYNA-Biotin Complex as follows:

(1) For each well strip to be used in the experiment (8-wells) prepare 500 µL by adding 5 µL of 100X KYNA-Biotin Complex to 495 µL Complex Diluent.

(2) Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

1X Avidin-HRP Conjugate

1. Prepare the 1X Avidin-HRP Conjugate immediately prior to use by diluting the 100X Avidin-HRP Conjugate1:100 with Conjugate Diluent as follows.
2. Briefly and gently mix the 100X Avidin-HRP Conjugate prior to pipetting.
3. For each well strip to be used in the experiment (8-wells) prepare 1,000 µL 1X Avidin-HRP Conjugate by adding 10 µL of 100X Avidin-HRP Conjugate to 990 µL Conjugate Diluent.
4. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

1X Wash Buffer

1. If crystals have formed in the 30X Wash Buffer concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.
2. Add the entire 20 mL contents of the 30X Wash Buffer bottle to 580 mL of ultra-pure water to a clean> 1,000 mL bottle or other vessel.
3. Seal and mix gently by inversion. Avoid foaming or bubbles.
4. Store the 1X Wash Buffer at room temperature until ready to use in the procedure. Store the prepared 1X Wash Buffer at 4°C for no longer than 1 week. Do not freeze.

Microplate Preparation

1. Micro-plates are provided ready to use and do not require rinsing or blocking.
2. Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
3. Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

Assay Procedure

Assay notes:

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results in inter- intra- assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates or fibrin strands or which are hemolytic of lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• For optimal control of small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at 37°C.

1. Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.
2. Retain at least one well as an absolute Blank without any samples or reagents.
3. Add 50 µL of serially titrated standards, diluted samples or blank into wells of the Anti-QA Microplate. At least two replicates of each standard, sample or blank is recommended.
4. Immediately add 50 µL of 1X KYNA-Biotin Complex to each well (excluding absolute Blank).
5. Cover the plate with the plate sealer and incubate at 37°C for 60 minutes.
6. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
7. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
8. Wash plate four times with 1X Wash Buffer as follows:

(1) Add 350 µL of 1X Wash Buffer to each assay well.

(2) Incubate for 2 minutes.

(3) Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

(4) Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

(5) Repeat steps (1) through (4) three more times.

9. Add 100 µL of 1X Avidin-HRP Conjugate to each well.
10. Cover the plate with the plate sealer and incubate at 37°C for 30 minutes.
11. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.
12. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
13. Repeat wash as in step 8 for a total of 5 washes.
14. Add 90 µL of TMB Substrate to each well, cover with plate sealer and incubate at 37°C in the dark for 10-20 minutes. Wells should change to gradations of blue. If the color is too deep based on the standard, adjust incubation times. (NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the bottom four standard wells, while the remaining standards still appear clearer.)
15. Add 50 µL of Stop Solution to each well. Well color should change to gradations of yellow immediately. Add the Stop Solution in the same well order as done for the TMB Substrate.
16. Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 15. If wavelength correction is available, set to 540 nm or 570 nm.

Calculation

For analysis of the assay results, calculate the Relative OD_{450} for each test or standard well as follows:

(Relative OD_{450}) = (Well OD_{450}) – (Mean Blank Well OD_{450})

The standard curve is generated by plotting the mean replicate Relative OD_{450} of each standard serial dilution point vs. the respective standard concentration. The KYNA concentration contained in the samples can be interpolated by using linear regression of each mean sample Relative OD_{450} against the standard curve. This is best achieved using curve fitting software.

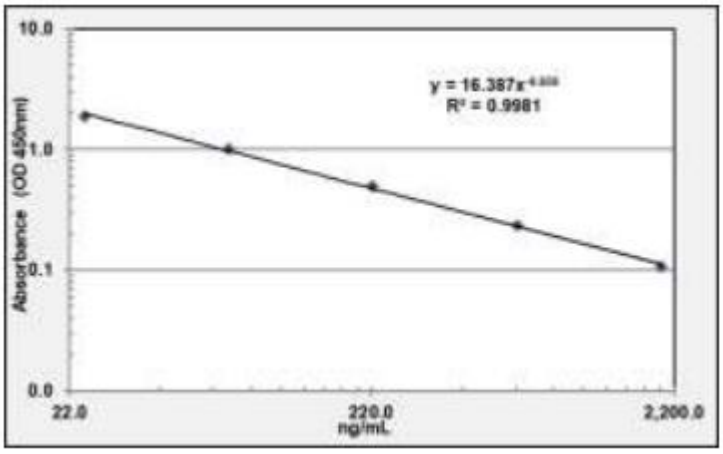
Note: if wavelength correction readings are available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

Typical Standard Curve

This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.

ng/mL	Absorbance		Mean Absorbance	Log of Concentration
	Rep 1	Rep 2		
2,000	0.107	0.119	0.113	1.393
666.67	0.235	0.237	0.236	1.870
222.22	0.498	0.494	0.496	2.347
74.07	1.011	1.007	1.009	2.824
24.69	1.891	1.883	1.887	3.301



Precision

Intra-assay reproducibility was evaluated with 20 replicates of 3 samples representing low, middle and high level target. Inter-assay reproducibility was evaluated with 3 samples representing low, middle and high level target using 8 replicates on each of 3 plates.

Sample	Intra-Assay			Inter-Assay		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/ml)	69.91	275.43	938.97	75.21	289.56	977.43
SD	4.964	14.873	48.826	5.415	17.663	52.781
CV (%)	7.1	5.4	5.2	7.2	6.1	5.4

Detection Range

24.69 - 2,000 ng/mL

Detection Limit

< 9.13 ng/mL (Derived by linear regression of OD450 of the Mean Blank + 2xSD)

Specificity

No detectable cross-reactivity with other relevant proteins.

Linearity

Linearity of the kit was evaluated by spiking KYNA into matrices, serially diluted and measured. Observed values were compared to the expected measurements.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	95-102%	86-96%	93-105%	80-97%
EDTA Plasma (n=5)	83-98%	82-93%	88-103%	94-101%
heparin Plasma (n=5)	85-95%	79-92%	81-96%	91-99%

Recovery

Matrices were spiked with KYNA and recovery rates were calculated by comparing the measured values to the expected concentrations.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	80-97	85
EDTA plasma (n=5)	82-102	92
Heparin Plasma (n=5)	83-99	94

Precautions

1. Read instructions fully prior to beginning use of the assay kit.
2. Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
3. Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
4. For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
5. Kit cannot be used beyond the expiration date on the label.