Human Cotinine GENLISA™ ELISA

REF : KBD9001

Ver 1.0

IVD

Enzyme Immunoassay for the Quantitative Determination of Cotinine in human serum and urine.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF **KBD9001** **Σ 96 tests**

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Introduction:

Cotinine derives from nicotine that in turn, derives from the dried leaves of *Nicotania tobacum*. Once absorbed in the body, nicotine rapidly metabolizes to cotinine and other metabolites which cannot be detected in significant quantity in the urine. Cotinine, on the other has a much longer half-life and is the principal urinary metabolite.

Intended Use:

The Human Cotinine GENLISA™ ELISA is intended for the quantitative determination of Cotinine in human serum and urine.

Principle:

Human Cotinine GENLISA™ ELISA method is a quantitative determination based on competitive enzyme-linked immunosorbent assay (ELISA) to determine the level of Cotinine molecules present in human serum and urine. Samples/Standards are added to the microtiter well which is pre-coated with Cotinine antibody. Enzyme conjugate is added to the microplate to compete with the Cotinine molecules present in the samples to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (A and B) is added to microwells and color develops proportionally to the amount of Cotinine molecules present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (96 wells) - 1 no
2. Standards (0.5 ml) - 0, 5, 10, 25, 50, 100 ng/ml
3. Enzyme Conjugate - 6.5 ml
4. (40X) Wash Buffer - 20 ml
5. TMB Substrate - 12 ml
6. Stop Solution - 12 ml
7. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Urine: Fresh Urine Sample should be used. Dilutions should be made if required.

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 2.5ml of Wash Buffer (40X) to 97.5ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.

2. Add **10 ul Standards/Sample** into appropriate wells.

3. Add **100 ul Enzyme Conjugate**. Incubate at 37ºC for 60 minutes.

4. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of 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.

5. Add **100 ul** of **TMB Substrate** into each well except blank well.

6. Incubate at 37ºC for 10 minutes.

7. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Interpretation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Cotinine concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Cotinine Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4-PL or a polynomial curve (2nd order) is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Positive Results:

If the determination value is higher or lower than normal range, it means there is an abnormal result. The final result should be diagnosed in correlation with the clinical symptoms and other diagnostic methods

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:

Sensitivity:
Assay sensitivity based on the minimum Cotinine concentration required to produce a three standard deviation from the assay is 1ng/ml.

Specificity:
The specificity based on this Cotinine ELISA was determined by generating inhibition curves for each of the compounds listed below the antisera cross reactivity below.

Compound	Approx. ng/ml equivalent to 100ng	Cross-Reactivity
Cotinine	100	100
Nicotine	>10000	<1
Nicotinamide	>10000	<1
Nicotinic Acid	>10000	<1

Safety Precautions:

- **This kit is For Invitro Diagnostic 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.

LIMITED WARRANTY

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THANK YOU FOR USING KRISHGEN PRODUCT!

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Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	All reagents should be allowed to reach room temperature before use.
2	Add **10 ul Standards/Sample** into appropriate wells
3	Add **100 ul** of **Enzyme Conjugate**. Incubate at 37ºC for 60 minutes.
4	Aspirate and **wash plate 5 times** with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of 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.
5	Add **100 ul** of **TMB Substrate** into each well except blank well.
6	**Incubate** at **37ºC** for **10 minutes**.
7	Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

MTP	Coated Microtiter Plate (8x12 wells)
STD	Standard
ENZY CONJ	Enzyme Conjugate
40X WASH BUF	(40X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature