Rat Creatinine (CR) ELISA

Cat No: K11-0308

ver1.1

Principle:
The Rat Creatinine (CR) ELISA is competitive enzyme-linked immunosorbent assay (ELISA) to assay the level of CR in samples. Standards or Samples competes with the biotinylated CR to form a complex with the CR antibody coated on microtiter well. Wells are washed to remove the excess conjugate and Streptavidin:HRP Conjugate is added to the microplate and incubated. After incubation and a washing step TMB Substrate is added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the CR in the samples is inversely proportional to the yellow color developed (absorbance) in the wells.

Intended Use:
This Kit is used to assay the level of Rat Creatinine (CR) in serum and plasma and other biological samples. The Kit is For Laboratory / Research Use Only.

Materials provided in the Kit:
1. Anti-CR Coated Microtiter Plate (96 wells) - 1 no
2. CR Standard (lyophilized, concentrated, 1000 ug/ml) - 2 vials
3. Biotinylated CR (lyophilized, concentrated) - 1 vial
4. Streptavidin:HRP Conjugate (concentrated) - 120 ul
5. Reagent Diluent – 300 ul
6. Standard Diluent - 20 ml
7. Biotin Antigen Dilution Buffer - 12 ml
8. HRP Conjugate Dilution Buffer - 12 ml
9. (20X) Wash Buffer - 25 ml
10. TMB Substrate - 12 ml
11. Stop Solution - 12 ml
12. 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. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator
8. Timer.

Storage Information:
1. For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Biotinylated Antigen, HRP Conjugate and 96-well plate should be stored at -20°C upon receipt while the others should be at 2-8°C.
2. For opened kits: Once the kit is opened, the remaining reagents still need to be stored according to the above storage conditions. In addition, return the unused wells to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.
3. All the reagents and wash solutions are stable until the expiration date of the kit.
4. 20 minutes prior before use, bring all components to room temperature (18-25ºC). Store all the components of the kit at its appropriate storage condition after use.
5. 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 samples in accordance with NCCLS regulations.

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

1. **Serum-** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
2. **Plasma-** Collect plasma using EDTA-Na2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
3. **Tissue Homogenates-** As hemolysis blood has relation to the assay results, it is necessary to remove residual blood by washing tissue with pre-coating PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue Normal 9 ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer or ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disruptor or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000xg to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg,
4. **Cell Culture Supernatant-** Centrifuge supernatant for 20 minutes at 1000xg at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
5. **Cell Culture Lysate-** Commercial RIPA kits are recommended to follow the instructions provided. Generally 0.5 ml RIPA lysis buffer would be appropriate to 2x10(6) cells, DNA must be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
6. **Other Biological Fluids-** Centrifuge samples for 20 minutes are 1000xg at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for 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) 500; dilute 25 ml of (20X) Wash Buffer in 475 ml of DI water.**
4. **Biotinylated CR Working Solution** - Reconstitute Biotinylated CR with 150 ul of Reagent Diluent, keep at room temperature for 10 minutes, gently shake. Dilute to the working concentration 100-fold with Biotin Antigen Dilution Buffer.
5. **Streptavidin HRP Conjugate Working Solution -** Briefly spin or centrifuge the Streptavidin:HRP Conjugate before use. Dilute to the working concentration 100-fold with HRP Conjugate Dilution Buffer.
6. **Standards Preparation**: Reconstitute original CR Standard with 1.0 ml of Standard Diluent. Keep the standard for 10 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
1000 ug/ml	Original Standard	Reconstitute with 1.0 ml Standard Diluent
200 ug/ml	Standard No.6	180 ul Standard Provided (1000 ug/ml) + 720 ul Standard Diluent
66.67 ug/ml	Standard No.5	300 ul Standard No.6 + 600 ul Standard Diluent
22.22 ug/ml	Standard No.4	300 ul Standard No.5 + 600 ul Standard Diluent
7.41 ug/ml	Standard No.3	300 ul Standard No.4 + 600 ul Standard Diluent
2.47 ug/ml	Standard No.2	300 ul Standard No.3 + 600 ul Standard Diluent
0 ug/ml	Standard No.1	300 ul Standard Diluent only

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 Rat Creatinine (Cr). High Dose Hook Effect is due to excess of antibody for very high concentrations of Rat Creatinine (Cr) present in the sample.
3. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Rat Creatinine (Cr).
4. It is recommended that all Standards and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to poor sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **50 ul prepared Standards and Samples** to respective wells.

3. Pipette **50 ul Biotinylated CR Antigen Working Solution** to all wells

4. Cover the plate with a sealer and incubate for 60 minutes at 37°C.

5. Aspirate and wash plate 4 times with diluted Wash Buffer (1X) 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.

6. Pipette **100 ul Streptavidin:HRP Conjugate Working Solution** to all wells. Mix well.

7. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

8. Aspirate and wash as per Step (5) above.

9. Pipette **100 ul TMB Substrate** in all the wells.

10. Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision.

11. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

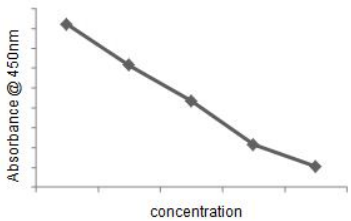
12. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Use the Net Absorbance (Absorbance of Standard/Sample - Absorbance of Blank) to calculate the Mean Absorbances. Using standard graph paper, plot the average value (absorbance 450nm) 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 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 concentration. If samples were diluted, multiply by the appropriate dilution factor.

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

Typical Graph



Precautions:
Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Performance Characteristics:
Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:
Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be **0.99 ug/ml**.

Specificity:
This assay has high sensitivity and excellent specificity for detection of CR. No significant cross-reactivity or interference between CR and analogues was observed.

Recovery
Matrices listed below were spiked with certain level of CR and the recovery rates were calculated by comparing the measured value to the expected amount of CR in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	79-90	85
EDTA Plasma(n=5)	85-96	89
Heparin Plasma(n=5)	82-103	90

Assay Range:
2.47 ug/ml – 200 ug/ml

Precision:
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Linearity:
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of CR and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8	1:16
serum (n=5)	92-103%	85-93%	79-91%	84-96%
EDTA plasma (n=5)	80-94%	92-105%	86-103%	80-93%
heparin plasma (n=5)	82-98%	78-95%	81-97%	87-102%

LIMITED WARRANTY

KinesisDx does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by KinesisDx, or against damages resulting from such non-KinesisDx made products or components. KinesisDx passes on to customer the warranty it received (if any) from the maker thereof of such non- KinesisDx made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by KinesisDx.

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ASSAY PROCEDURE

1	Bring all reagents to room temperature before use.		
2	Pipette Standards 1 - 6 Samples	50 ul	50 ul
4	Pipette Biotinylated CR	50 ul	50 ul
5	Incubate	60 minutes (37ºC)	
6	Wash 1X Wash Buffer	Decant, 4 x 300 ul	
7	Pipette Streptavidin: HRP Conjugate	100 ul	100 ul
9	Incubate	30 minutes (37ºC)	
10	Wash 1X Wash Buffer	Decant, 4 x 300 ul	
11	Pipette TMB Substrate	100 ul	
12	Incubate in the dark	10 minutes (37ºC)	
13	Pipette Stop Solution	100 ul	
14	Measure 450 within 15 mins		