Creatinine Competitive ELISA Kit

Catalog Number EEL159 (96 tests)
Rev 2.0

For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Creatinine (Cr) Immunoassay Kit is a solid-phase Competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of Cr in serum, plasma, and other biological fluids independent of species.
Creatinine is a waste product from protein in the diet and from the muscles of the body. Creatinine is removed from the body by the kidneys; as kidney disease progresses, the level of creatinine in the blood increases.

Contents and storage

An unopened kit can be stored at 2-8℃ for 12 months. After opening, store the items separately according to the conditions specified in the table below.
Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution. The volume of reagents in partial shipments is slightly more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Components	Quantity (96 tests)	Storage
Cr Antigen Coated Microplate	8 wells x 12 strips	-20℃, 12 months
Cr Standard	2 vials	
Cr Biotinylated Detection Antibody (100X)	120 µL	
HRP Conjugate (100X)	120 µL	-20℃ (Protect from light), 12 months
Standard & Sample Diluent	20 mL	2-8℃, 12 months
Biotinylated Detection Antibody Diluent	14 mL	
HRP Conjugate Diluent	14 mL	
Wash Buffer Concentrate (25X)	30 mL	
Substrate Reagent	10 mL	2-8℃ (Protect from light), 12 months
Stop Solution; contains 1 M H_2SO_4, **CAUSTIC**	10 mL	2-8℃, 12 months
Plate Sealer	5	

Required materials

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions
- Incubator capable of maintaining 37℃.

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Sample preparation guidelines

Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃ before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Plasma: Collect plasma using EDTA or heparin (EDTA-Na_2 is most recommended) as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.

Other biological fluids: Centrifuge samples for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.

Note:

- Collect samples in pyrogen/endotoxin-free tubes.
- Samples should be assayed within 7 days when stored at 2-8℃, otherwise samples must be aliquoted and stored at -20℃ (≤ 1 month) or -80℃ (≤ 3 months). Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge, or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, the optimal dilution for each application should be determined (It is recommended to carry out the preliminary test referring to the expected values of samples on page 8).

Use all prepared samples within 2 hours of dilution. It is not recommended to conduct experiments after 2 hours.

Prepare 1X Wash Buffer

1. Dilute 30 mL of Wash Solution Concentrate (25X) with 720 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer at 2-8℃. Use the diluted buffer within 3 months.

Note: if crystals have formed in the concentrate, warm it in a 40℃-water bath and mix it gently until the crystals have completely dissolved.

Prepare 1X Biotinylated Detection Antibody Solution

Note: The working solution should be prepared just before use

1. Calculate the required amount before the experiment (50 μL/well). In preparation, slightly more than calculated should be prepared.
2. Centrifuge the Concentrated Biotinylated Detection Ab at 800×g at 2-8℃ for 1 min.
3. Dilute the Concentrated Biotinylated Detection Ab (100X) to 1X working solution with Biotinylated Detection Ab Diluent.

Prepare 1X HRP Conjugate Solution

Note: The working solution should be prepared just before use

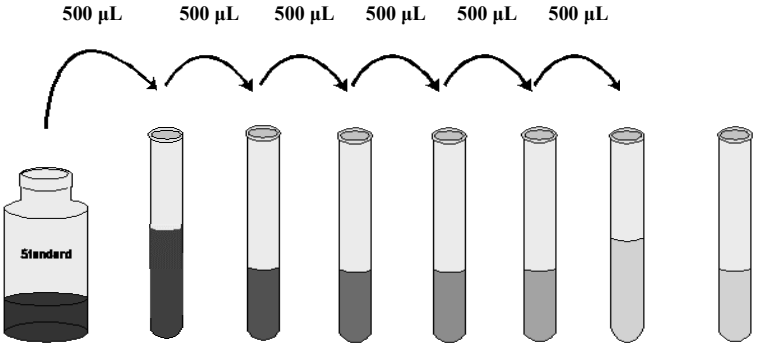
1. Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared.
2. Centrifuge the Concentrated HRP Conjugate at 800×g at 2-8℃ for 1 min.
3. Dilute the Concentrated HRP Conjugate (100X) to 1X working solution with HRP Conjugate Diluent.

Prepare diluted standards

Note: Use glass or plastic tubes for diluting standards.

1. Centrifuge the standard at 10,000×g at 2-8℃ for 1 min to ensure the contents are at the bottom of vial.
2. Add 1 mL of Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. Once fully dissolved, mix it thoroughly with a pipette. This reconstitution produces a working solution of 80 μg/mL.
3. Take 7 tubes, add 500 μL of Standard & Sample Diluent to each tube. Pipette 500 μL of the 80 μg/mL working solution to the first tube and mix up to produce a 40 μg/mL working solution. Pipette 500 μL of the solution from the former tube into the latter one according to this step. The last tube is regarded as a blank, don't pipette solution into it from the former tube. The recommended dilution gradient is as follows: 80, 40, 20, 10, 5, 2.5, 1.25, 0 μg/mL.

4. The illustration of diluted standards below is for reference. Mix thoroughly between dilution gradients.



Diluent Volume	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
Std1	**Std2**	**Std3**	**Std4**	**Std5**	**Std6**	**Std7**	**Std8**
80 µg/mL	**40 µg/mL**	**20 µg/mL**	**10 µg/mL**	**5 µg/mL**	**2.5 µg/mL**	**1.25 µg/mL**	**0 µg/mL**

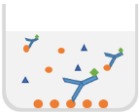
5. Use the diluted standards within 2 hours of preparation. If multiple standard tests are to be carried out, the redissolved standard solution (standard with the highest concentration) can be divided into 2-3 vials and frozen to -20℃, to be used within half a month. Avoid multiple freeze-thaw cycles.

Perform ELISA (Total assay time: 2 hours)

IMPORTANT! Perform a standard curve with each assay.

Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at -20℃ for future use. The silica pack in the bag keeps the plate dry.



1. Add antigen and biotinylated detection antibody

Note: solutions should be added to the bottom of the ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

a. For the standard curve, add 50 µL of standards to the appropriate wells. For samples, add 50 µL of pretreated samples to the wells.

b. Immediately add 50 µL of **Biotinylated Detection Ab working solution** to each well.

c. Cover the plate with plate sealer and incubate for 45 min at 37℃.

d. Thoroughly aspirate the solution and wash wells 3 times with 350 µL of 1X Wash Buffer. Decant the solution from each well, add 350 µL of **wash buffer** to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Proceed immediately to the next step, making sure the wells do not dry out.

2. Add HRP conjugate

a. Add 100 µL **HRP Conjugate Working Solution** into each well.

b. Cover the plate with plate sealer and incubate for 30 min at 37℃.

c. Thoroughly aspirate the solution and repeat the wash process for 5 times as conducted in step 1.

3. Add substrate

a. Add 90 µL **Substrate Reagent** to each well.

b. Cover the plate with plate sealer and incubate for about 15 min at 37℃. Protect the plate from light.

Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min.

4. Add stop solution

a. Add 50 µL **Stop Solution** to each well. This step should be done in the same order as the substrate solution. Tap the side of the plate gently to mix.

b. The solution in the wells will change from blue to yellow.

Read the plate and generate the standard curve

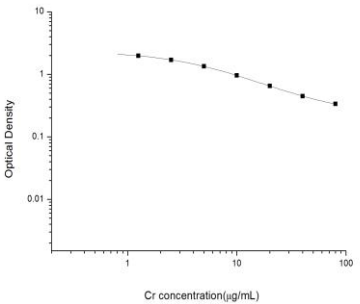
1. Preheat the Microplate Reader for about 15 min before OD measurement.
2. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
3. Use curve-fitting software to generate the standard curve. A four-parameter algorithm provides the best standard curve fit.
4. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Note: If the OD of the sample under the lowest limit of the standard curve, you should re-test it with an appropriate dilution.

Performance characteristics

▪ Standard curve (example)

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data over the range of 0–80 ng/mL Cr is provided below for reference only.

Concentration (μg/mL)	OD at 450 nm	Standard Curve for Cr
80	0.334	
40	0.448	
20	0.648	
10	0.954	
5	1.334	
2.5	1.702	
1.25	1.981	
0	2.351	

- Inter-assay Precision

Three Human serum samples with low, medium, and high level Cr were tested 10 times in duplicate to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (µg/mL)	4.2	11.1	31.6
Standard deviation	0.3	0.5	1.7
%CV	7.14	4.5	5.38

CV = Coefficient of Variation

- Intra-assay Precision

Three Human serum samples with low, medium, and high level Cr were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (µg/mL)	3.9	10.7	34.8
Standard deviation	0.2	0.5	1.8
%CV	5.13	4.67	5.17

CV = Coefficient of Variation

- Expected values

Sixteen random Human serum/plasma samples were tested in the assay.

Sample Type	**Cr** Range (**µg/mL**)	**Cr** Average (**µg/mL**)
Serum (n=16)	2.41-8.33	5.21
Plasma (n=16)	1.97-11.2	6.64

▪ Recovery

The recovery of Cr spiked at three different levels in Human samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	88-100	94
EDTA plasma (n=8)	92-108	100
Cell culture media (n=8)	94-107	99

▪ Linearity of dilution

Human Samples were spiked with high concentrations of Cr and diluted with Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)	Cell culture media(n=5)
1:2	Range (%)	91-104	94-110	93-107
	Average (%)	97	101	100
1:4	Range (%)	89-102	91-102	96-110
	Average (%)	95	97	102
1:8	Range (%)	83-96	94-106	92-109
	Average (%)	90	100	100
1:16	Range (%)	90-105	92-106	94-108
	Average (%)	96	97	102

- Specificity

This assay has been shown to detect Cr only from human, mouse, rat, monkey, rabbit and porcine samples. Please contact technical support for cross-reactivity information on other species.

- Sensitivity

The analytical sensitivity of the assay is 0.75 µg/mL Cr. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times and calculating the corresponding concentration.

Limited product warranty

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