# Cr (Creatinine) colorimetric Assay Kit

**货号:** EU3134

**规格:** 48T/96T

**适用种属:** Universal

**范围:** 25-800μmol/L

**灵敏度:** 10μmol/L

**应用:** 用于定量检测血清、血浆、尿液中的肌酐含量。

**储存条件:**2-8°C

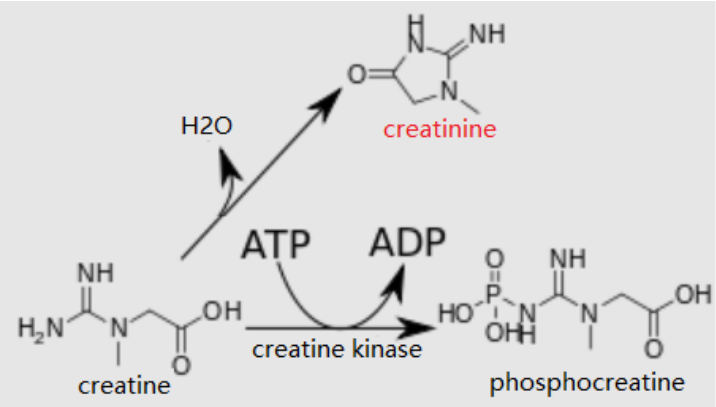
**保质期:** 见试剂盒标签

**实验方法:** 酶催化法

**注: 仅供科研使用**

## 背景资料

肌酸是磷酸肌酸(p-肌酸)的代谢物，磷酸肌酸是一种用于储存高能量磷酸盐的分子，组织可以利用磷酸腺苷产生 ATP。 肌酸可来自于饮食，或由精氨酸、甘氨酸和蛋氨酸合成。 这个过程发生在肾脏和肝脏，在其他器官系统和不同物种可能存在差异。 肌酸和 p 肌酸以非酶的方式转化为代谢产物肌酸，扩散到血液中并由肾脏排泄。 在体内，这种转化似乎是不可逆的，而在体外，较高的温度和较低的 PH 值对它有利。肌酸由肌酸自发形成，在正常条件下，它的形成以相对恒定的速率发生。 每天个体内肌酐水平的变化<15%，使其成为尿液中发现的其他分子水平正常化的有用标记。 肌酐水平的改变可能与导致肾血流量下降的情况有关，如糖尿病和心血管疾病。




## 试剂盒组件:

| No. | Item | Specifications (48T/96T) | Storage |
|---|---|---|---|



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| | | | |
|---|---|---|---|
| **E001** | ELISA Microplate | 8×6/8×12 | |
| **E002** | Lyophilized Standard(1000nmol/vial) | 1vial/2vial | 2-8°C/-20°C |
| **E003** | Enzyme Solution A | 12ml/25ml | 2-8°C (Avoid Direct Light) |
| **E004** | Enzyme Solution B | 4ml/8ml | 2-8°C (Avoid Direct Light) |
| **E006** | Plate Sealer | 1/2pieces | |
| **E007** | Product Description | 1copy | |

## 实验数据及标准曲线

下表展示的实验数据，是 Cr 试剂盒在本公司实验室环境条件下，依说明书标准操作所得，仅供参考。因具体实验环境及操作存在差异，用户需根据自身实验获得标准曲线，不建议直接套用以下实验数据。((OD546))

| STD.(umol/L) | OD-A2 | OD-A1 | OD-(A2-A1) | Corrected |
|---|---|---|---|---|
| 0 | 0.038 | 0.036 | 0.002 | 0 |
| 25 | 0.051 | 0.04 | 0.011 | 0.009 |
| 50 | 0.058 | 0.038 | 0.02 | 0.018 |
| 100 | 0.079 | 0.039 | 0.04 | 0.038 |
| 200 | 0.111 | 0.04 | 0.071 | 0.069 |
| 300 | 0.155 | 0.039 | 0.116 | 0.114 |
| 600 | 0.257 | 0.039 | 0.218 | 0.216 |
| 800 | 0.343 | 0.039 | 0.304 | 0.302 |



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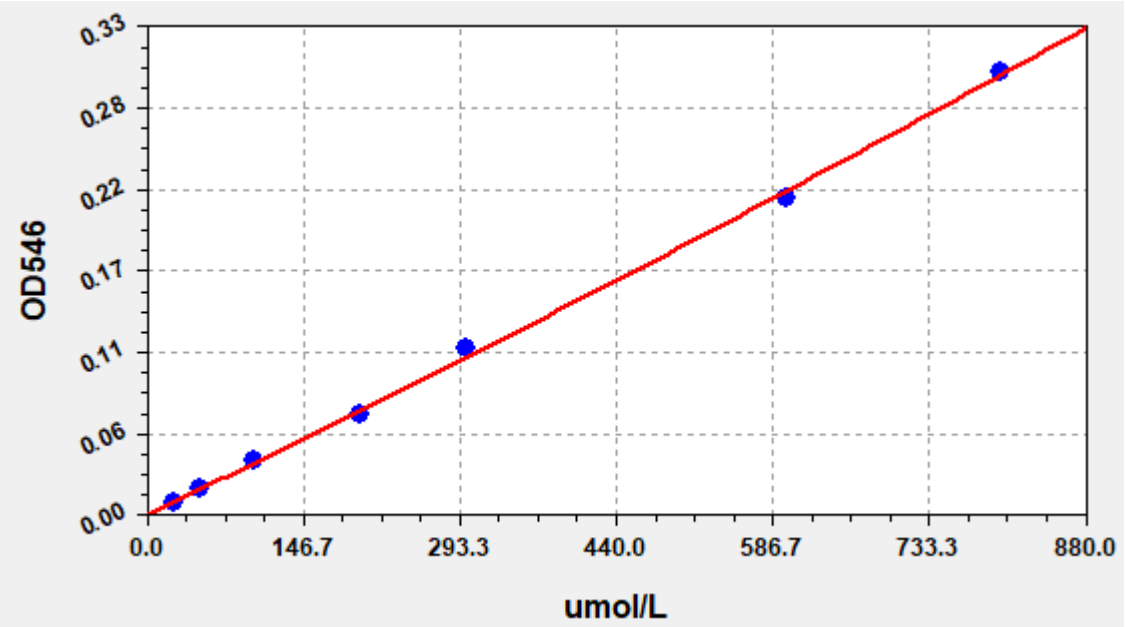


## 特异性和抗干扰

该方法对肌酐的检测灵敏度高，特异性好。 肌酐和类似物之间没有明显的交叉反应或干扰。

维生素 C≤500mg/L，血红蛋白≤1g/L，乳糜≤0.30%，

胆红素≤342 umol / L(干扰< 10%)

## 回收率

将一定含量的肌酐掺入下表列出的样本中，并通过将测量值与样品中肌酐的预期量进行比较来计算回收率。

| Matrix | Recovery Range (%) | Average (%) |
|---|---|---|
| Serum(n=5) | 91-102 | 95 |
| EDTA Plasma(n=5) | 86-98 | 95 |
| Heparin Plasma(n=5) | 92-100 | 96 |
| urine(n=5) | 95-103 | 97 |

## 线性范围

线性相关 (20-2000)umol/L,R≥0.990

偏离线性(20-70)umol/L, AD≤7umol/L，(70-2000)umol/L, AD≤10umol/L，



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## 精密度

批内差: CV<4%

批间差: CV<6%

## 稳定性

本试剂盒储存在 2-8°C(避免阳光直射)，不得冷冻及反复冻融。 该产品有效期为 6 个月。 开封后，请避免污染，在 2-8°C 保存，可稳定 30 天。

## 样品数据

人血清(60-120umol/L)



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## 实验原理

肌酐经肌酐酶催化生成肌酸，肌酸经肌酐酶催化生成肌氨酸和尿素，肌氨酸经肌氨酸氧化酶催化生成甘氨酸、甲醛和过氧化氢。 过氧化氢与显色剂在过氧化物酶催化下反应生成粉红色化合物，在 546 nm(主波长)/700 nm(副波长)的 OD 值计算肌酐含量。

## 注意事项

1. 为检验实验操作的有效性和样品稀释比例的适宜性，建议使用标准品和少量样品进行预实验。
2. 在使用该试剂盒之前，将试剂离心至管底。
3. 所有试剂避光贮存。
4. 建议对标准品和样品进行重复分析。
5. 不要重复使用吸头和试管，以避免交叉污染。
6. 避免同时使用不同批次的试剂。

## 试剂盒组件外所需器材和试剂

1. 酶标仪（波长：546nm/700nm）
2. 37°C 恒温箱
3. 漩涡混匀器
4. 精密的单道和多道移液器以及干净的一次性枪头
5. 干净的 EP 管
6. 去离子水或蒸馏水

## 样品采集和储存（通用）

- **血清**：将所有的血清样品在室温下放置 2 小时或在 2-8°C 下放置过夜，并以大约 1000×g 离心 20 分钟，收集上清液并立即进行测定。采血管要求是一次性的，且不含热原和内毒素。
- **血浆**：使用 EDTA-$Na_2$ 或肝素作为抗凝剂采集血浆。采集后 30 分钟内于 2-8°C 以 1000×g 离心 15 分钟，收集上清液并立即进行测定。应避免溶血及含高胆固醇的样品。
- **尿液**：在 2-8°C 下以 1000×g 的速度离心培养上清液 20 分钟。收集澄清的上清液并立即进行测定或分装冻存。

**注意：**5 天内使用的样品可以在 2-8°C 下保存。否则样品必须在-20°C（使用时间在 1 个月内）或-80°C（使用时间在 1-2 年内）下保存，尽量避免待测蛋白的生物活性的丧失与样品的污染。避免多次反复冻融样品。溶血严重的样品不适用于该测定实验。

## 样品稀释

用户应预估检测样品中肌酐的浓度，选择合适的稀释比例，使稀释后的目的物浓度落在试剂盒的最佳检测范围内。 用蒸馏水稀释样品，可能需要多次试验。测试样品必须与蒸馏水充分混合，并在预实验中制作标准曲线。



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## 试剂准备与储存

使用前，将所有试剂和样品置于室温平衡 20 分钟。

**1, 样品准备:**

| 样品 | 稀释比 |
|---|---|
| Serum(human, mouse, rat, pig, dog, goat) | 不稀释 |
| plasma(human, mouse, rat, pig, dog, goat) | 不稀释 |
| urine(human, mouse, rat) | 1/20 ~ 1/30 稀释 |

**2, 标准品:**

稀释后的标准品溶液不稳定，必须在 2 小时内使用。

1、在标准品管中加入 1ml 蒸馏水(标记为原液)，室温静置 10 分钟，然后充分混匀。

2、参考以下表格配置各梯度标准品（1-7）。

| Standard # | concentration (umol/L) | stock (1000umol/L) (ul) | $H_2O$ (ul) | final volume(ul) |
|---|---|---|---|---|
| 1 | 800 | 80 | 20 | 100 |
| 2 | 600 | 60 | 40 | |
| 3 | 300 | 30 | 70 | |
| 4 | 200 | 20 | 80 | |
| 5 | 100 | 10 | 90 | |
| 6 | 50 | 5 | 95 | |
| 7 | 25 | 2.5 | 97.5 | |
| blank | 0 | 0 | 100 | |

**以下提供了两种检测方法，请自行选择，结果供参考。**

## 分析程序(标准操作程序)

1. 在板上分别设置标准品孔、样品孔，空白孔，并记录其位置。 建议对每个标准品和样品进行一式两份的测量。
2. 加标准品: 取标准品(1-7)各 10μl，加入标准品孔。空白孔加入 100ul 蒸馏水。
3. 加样: 将适当稀释后的样品 10μl 加入样品孔中。
4. 加入酶溶液 A: 每孔加入酶溶液 A 210μl，充分混匀。



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5. 孵育: 盖上覆膜，37° C 孵育 5 分钟。

6. OD546 测量: 取下覆膜，立即用酶标仪读取 546nm 的 OD 值(标记 A1)

7. 加入酶溶液 B:每孔加入酶溶液 B 70μl，充分混匀。

8. 孵育: 盖上覆膜，37° C 孵育 5 分钟。

9. OD546 测量:取下覆膜，立即用用酶标仪读取 546nm 的 OD 值(标记 A2)

10. 计算:使用软件进行计算

关于计算，(相对 OD546) = (OD546 A2) - (OD546 A1))。 标准曲线可以绘制为各标准品溶液的相对 OD546 (Y)与各自标准溶液的浓度(X)之间的关系。可以从标准曲线中计算样品的目标浓度。 建议使用一些专业软件进行计算，如 Curve Expert 1.3 或 1.4。 (线性拟合 y=a + bx，)（操作视频：https://www.fn-test.cn/videos/standard-curve-drawing-video-in-elisa-kit/）

## 分析程序(简单操作程序)

1. 在板上分别设置原液标准品孔（Stock）、样品孔、对照孔(blank)，并记录其位置。 建议对每个标准和样品进行一式两份的测量。

2. 加入标准品: 取 10μl 原液标准品和空白品（蒸馏水）分别加入标准/空白孔中。

3. 加样:将 10μl 适当稀释后的样品加入待测样品孔中。

4. 加入酶溶液 A: 每孔加入酶溶液 A 210μl，充分混匀。

5. 孵育:盖上覆膜，37° C 孵育 5 分钟。

6. OD546 测量:取下覆膜，立即用酶标仪读取 546nm 的 OD 值(标记 A1)

7. 加入酶溶液 B:每孔加入酶溶液 B 70μl，充分混匀。

8. 孵育:盖上覆膜，37° C 孵育 5 分钟。

9. OD 测量:取下覆膜，立即用酶标仪读取 546nm 的 OD 值(标记 A2)

10. 计算

$$\text{Crea(umol/L)} = \frac{\text{Sample(A2-A1)}}{\text{Stock Standard(A2-A1)}} \times \text{Stock Standard concentration}$$

注:若所测样品被稀释，则计算得到的浓度需乘以稀释倍数，得到稀释前的浓度。



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# Cr (Creatinine) colorimetric Assay Kit

**Catalogue No.:** EU3134

**Size:** 48T/96T

**Reactivity:** Universal

**Range:** 25-800μmol/L

**Sensitivity:** 10μmol/L

**Application:** For quantitative detection of creatinine in serum, plasma and urine.

**Storage:** 2-8°C

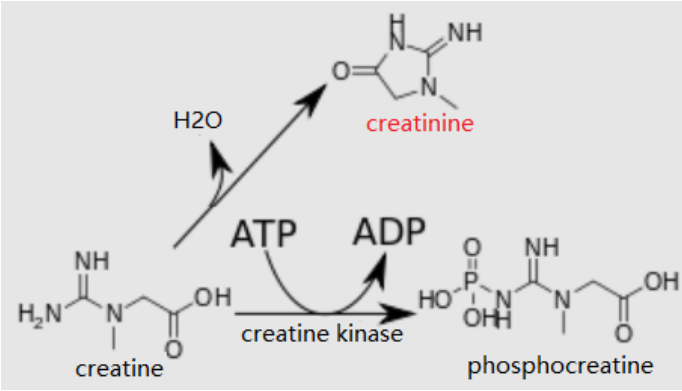
**Expiry Date:** see kit label

**Principle:** Enzyme catalysis

**NOTE: FOR RESEARCH USE ONLY.**

**1,BACKROUND**

Creatinine is a metabolite of phosphocreatine (p-creatine), a molecule used as a store for highenergy phosphate that can be utilized by tissues for the production of ATP. Creatine either comes from the diet or is synthesized from the amino acids arginine, glycine, and methionine. This occurs in the kidneys and liver, although other organ systems may be involved and species-specific differences may exist. Creatine and p-creatine are converted non-enzymatically to the metabolite creatinine, which diffuses into the blood and is excreted by the kidneys. In vivo, this conversion appears to be irreversible and in vitro it is favored by higher temperatures and lower pH. Creatinine forms spontaneously from pcreatine, and under normal conditions, its formation occurs at a relatively constant rate. Intra-individual variation of creatinine levels is <15% from day to day, making it a useful marker for normalizing levels of other molecules found in urine. Altered creatinine levels may be associated with conditions that result in decreased renal blood flow, such as diabetes and cardiovascular disease.



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**Kit Components**

| No. | Item | Specifications (48T/96T) | Storage |
|---|---|---|---|
| **E001** | ELISA Microplate | 8×6/8×12 | |
| **E002** | Lyophilized Standard(1000nmol/vial) | 1vial/2vial | 2-8°C/-20°C |
| **E003** | Enzyme Solution A | 12ml/25ml | 2-8°C (Avoid Direct Light) |
| **E004** | Enzyme Solution B | 4ml/8ml | 2-8°C (Avoid Direct Light) |
| **E006** | Plate Sealer | 1/2pieces | |
| **E007** | Product Description | 1copy | |

**Typical Data & Standard Curve**

Results of a typical standard operation of a creatinine are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves. (OD546)

| STD.(umol/L) | OD-A2 | OD-A1 | OD-(A2-A1) | Corrected |
|---|---|---|---|---|
| 0 | 0.038 | 0.036 | 0.002 | 0 |
| 25 | 0.051 | 0.04 | 0.011 | 0.009 |
| 50 | 0.058 | 0.038 | 0.02 | 0.018 |
| 100 | 0.079 | 0.039 | 0.04 | 0.038 |
| 200 | 0.111 | 0.04 | 0.071 | 0.069 |
| 300 | 0.155 | 0.039 | 0.116 | 0.114 |
| 600 | 0.257 | 0.039 | 0.218 | 0.216 |
| 800 | 0.343 | 0.039 | 0.304 | 0.302 |



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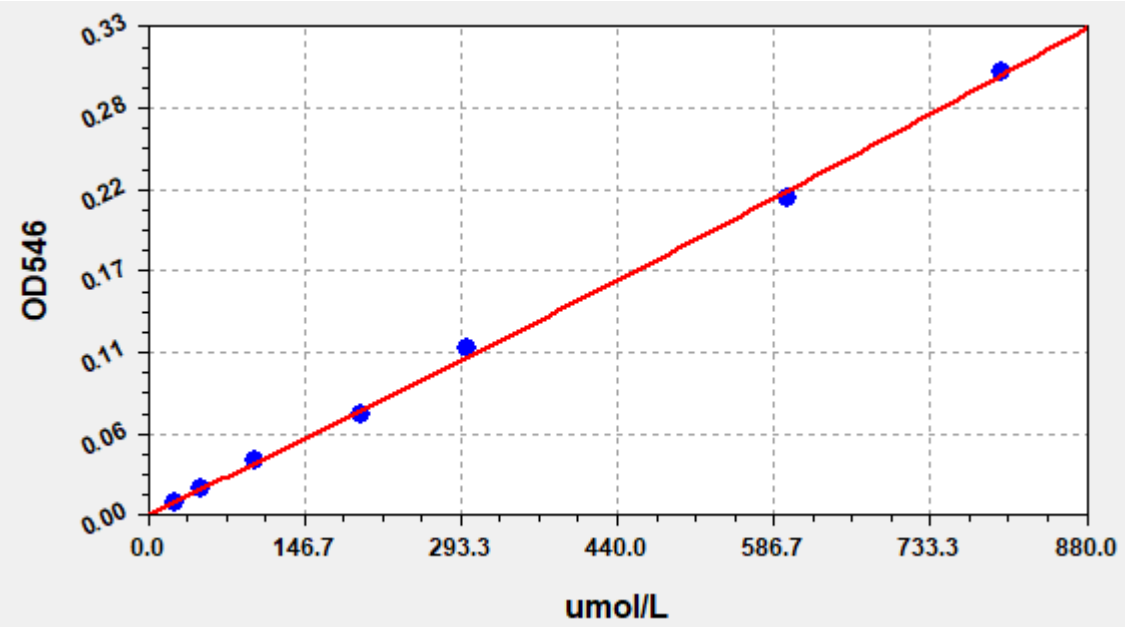


**Specificity & anti-interference**

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

Vitamin C ≤500mg/L , Hemoglobin ≤1g/L , Cchylechyle ≤0.30%,

Bilirubin ≤342umol/L(Interference < 10%)

**Recovery**

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

| Matrix | Recovery Range (%) | Average (%) |
|---|---|---|
| Serum(n=5) | 91-102 | 95 |
| EDTA Plasma(n=5) | 86-98 | 95 |
| Heparin Plasma(n=5) | 92-100 | 96 |
| urine(n=5) | 95-103 | 97 |

**Linear range**



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Correlation coefficient(20-2000)umol/L,R≥0.990

Deviation from linearity(20-70)umol/L, AD≤7umol/L，(70-2000)umol/L, AD≤10umol/L，

**Precision**

Intra-Assay: CV≤6% ,Inter-Assay: CV≤4%

**Stability**

The kit is stored at 2-8°C (Avoid Direct Light), and not be frozen or thawed. The product is valid for 6 months. After opening, store at 2-8°C, it can be stable for 30 days, avoid contamination.

**Sample data** (human serum：60-120umol/L,)



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# Operation Procedure

## Principle of the Assay

Creatinine is catalyzed by creatininase to form creatine, and creatine is catalyzed by creatinase to form sarcosine and urea, then sarcosine catalyzed by sarcosine oxidase to form glycine, formaldehyde and hydrogen peroxide. Hydrogen peroxide and chromogenic agent react under the catalysis of peroxidase to produce pink compound The creatinine content was calculated by ready the OD value at 546 nm(dominant wavelength)/700 nm (Deputy wavelength).

## Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
3. Storage all reagents avoid light.
4. Duplicate well assay is recommended for both standard and sample testing.
5. Don't reuse tips and tubes to avoid cross contamination.
6. Avoid using the reagents from different batches together.

## Material Required but Not Supplied

7. Microplate reader (wavelength:546nm/700nm)
8. 37°C incubator
9. Vortex mixer
10. Precision single and multi-channel pipette and disposable tips
11. Clean tubes and Eppendorf tubes
12. Deionized or distilled water.

## Sample Collection and Storage (universal)

- **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.
- **Plasma:** Collect plasma using EDTA-$Na_2$ 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.
- **Urine:** Centrifuge supernatant for 20 minutes at 1000×g at 2-8°C to remove insoluble impurity and cell debris. Collect the clear 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.



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## Sample Dilution

The user should estimate the concentration of the creatinine in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with distilled water, and several trials may be necessary. The test sample must be well mixed with distilled water. And also standard curves and sample should be making in pre-experiment.

## Reagent Preparation and Storage

Bring all reagents and samples to room temperature for 20 minutes before use.

**1, Sample prepare:**

| Sample | dilution ratio |
|---|---|
| Serum(human, mouse, rat, pig, dog, goat) | undiluted |
| plasma(human, mouse, rat, pig, dog, goat) | undiluted |
| urine(human, mouse, rat) | 1/20 ~ 1/30 dilute |

**2，Standard prepare:**

Diluted standard solution is unstable and must be used within 2 hours.

1, Add 1 ml distilled water into Standard tube (labeled as stock), keep the tube at room temperature for 10 minutes and mix them thoroughly.

2, Refer to the table to prepare the standard.

| Standard # | concentration (umol/L) | stock (1000umol/L) (ul) | $H_2O$ (ul) | final volume(ul) |
|---|---|---|---|---|
| 1 | 800 | 80 | 20 | 100 |
| 2 | 600 | 60 | 40 | |
| 3 | 300 | 30 | 70 | |
| 4 | 200 | 20 | 80 | |
| 5 | 100 | 10 | 90 | |
| 6 | 50 | 5 | 95 | |



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| | | | | |
|---|---|---|---|---|
| 7 | 25 | 2.5 | 97.5 | |
| blank | 0 | 0 | 100 | |

**The following two assay procedures are provided, please choose by yourself, the results are all for reference.**

## Assay Procedure(Standard operating procedure)

1. Set standard, test samples control (blank) wells on the plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. **Prepare Standards:** Aliquot 10µl of standard (1-7) and blank into the standard wells.
3. **Add Samples:** Add 10µl of properly diluted sample into sample wells.
4. **Add Enzyme Solution A:** Add 210µl of enzyme Solution A into each well and mix the well.
5. **Incubate:** Seal the plate with a cover and incubate at 37°C for 5 minutes.
6. **OD Measurement:** Remove the cover and read the O.D. absorbance at 546nm in Microplate Reader immediately(mark A1)
7. **Add Enzyme Solution B:** Add 70µl of enzyme Solution B into each well and mix the well.
8. **Incubate:** Seal the plate with a cover and incubate at 37°C for 5 minutes.
9. **OD Measurement:** Remove the cover and read the O.D. absorbance at 546nm in Microplate Reader immediately(mark A2)
10. **Calculation:** Use the software for calculation

    Regarding calculation, (the relative O.D.546) = (the O.D.546 A2 of each well) – (the O.D.546 A1 of each well)). The standard curve can be plotted as the relative O.D.546 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as **Curve Expert 1.3 or 1.4.(Linear Fit y=a + bx,** （Operate Video：https://www.fn-test.com/videos/elisa-sample-concentration-calculation/）)

## Assay Procedure(Simple operating procedure)

1. Set standard (Stock), test samples, control (blank) wells on the plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. **Prepare Standard:** Aliquot 10µl of Stock Standard and blank into the standard/blank wells.
3. **Add Samples:** Add 10µl of properly diluted sample into test sample wells.
4. **Add Enzyme Solution A:** Add 210µl of enzyme Solution A into each test well mix the well.
5. **Incubate:** Seal the plate with a cover and incubate at 37°C for 5 minutes.
6. **OD Measurement:** Remove the cover and read the O.D. absorbance at 546nm in Microplate Reader immediately(A1)
7. **Add Enzyme Solution B:** Add 70µl of enzyme Solution B into each test well mix the well.



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8. **Incubate:** Seal the plate with a cover and incubate at 37°C for 5 minutes.

9. **OD Measurement:** Remove the cover and read the O.D. absorbance at 546nm in Microplate Reader immediately(A2)

10. **Calculation:**

$$\text{Crea(umol/L)} = \frac{\text{Sample(A2-A1)}}{\text{Stock Standard(A2-A1)}} \times \text{Stock Standard concentration}$$

**Note:** If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.



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