Bio-Swamp
Customer Support, Product Safety & Technical Service

Human Cystatin C (CysC) ELISA Kit

Catalog Number: **HM10642**

- **For the quantitative in vitro determination of CysC concentrations in Human culture supernates, serum, plasma and tissue.**
- **Expiration date：six months .**
- **Storage： 2-8℃.**

INTENDED USE

An enzyme immunoassay quantitative measurement in cell culture in vitro Human CysC supernates, serum, plasma and tissue.

PRINCIPLE

The kit assay Human CysC level in the sample， use Purified Human CysC antibody to coat microtiter plate wells, make solid-phase antibody, then add CysC to wells, Combined CysC antibody which With HRP labeled , become antibody - antigen - enzyme- antibody complex, after washing Completely, Add TMB substrate solution,TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of CysC in the samples is then determined by comparing the O.D. of the samples to the standard curve.

WARNINGS AND PRECAUTIONS

- This kit is only for scientific research, and shall not be used as a clinical diagnosis of use.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21-26°C) before starting the test.

Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.

- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Avoid contact with Stop Solution containing 0.5 M H2SO4. It may cause skin irritation and burns.
- Some reagents contain Proclin, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets

MATERIALS PROVIDED WITH THE KIT

1.	**Instruction**	**1**
2.	**Closure Plate Membrane**	**2**
3.	**Microelisa Stripplate**	**12well×8strips**
4.	**Standard 240ng/ml**	**0.6ml×1 bottle**
5.	**Standard diluent**	**2ml×1 bottle**
6.	**Biotinylated anti –CysC–antibody**	**1.5ml×1 bottle**
7.	**Chromogen Solution A**	**6ml×1 bottle**
8.	**Chromogen Solution B**	**6ml×1 bottle**
9.	**Stop Solution**	**6ml×1 bottle**
10.	**HRP-Conjugate Reagent**	**6ml×1 bottle**
11.	**Wash Buffer Concentrate**	**(20ml×30 fold)×1bottle**

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 ml to 1 ml volumes.
- 100 ml and 1 liter graduated cylinders.
- Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
- Absorbent paper.
- 37°C incubator.
- Distilled or deionized water.
- Data analysis and graphing software. Graph paper: linear (Cartesian),log-log or semi-log, or log-logit as desired.
- Tubes to prepare standard or sample dilutions.

STORAGE CONDITIONS

- When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date.
- Do not use reagents beyond this date. Opened reagents must be stored at 2 °C to 8 °C.
- Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
- Opened kits retain activity for 8 weeks if stored as described above.

REAGENT PREPARATION

Bring all reagents to room temperature before use

SPECIMEN COLLECTION AND PREPARATION

1. **Serum**-Use a serum separator tube(SST) and allow samples to clot for 30minutes before centrifugation for 15minutes at approximately 1000 xg.Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.
2. **Plasma**-Collect plasma using EDTA or Citric acid sodium as an anticoagulant.Centrifuge samples for 15 minutes at 1000 x g at 2-8°C within 30minutes of collection. Store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.
3. **Cell culture fluid and other biological fluids**-Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C.Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

◆ General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Determine absorption with an ELISA reader at 450 nm against 630 nm as reference. If no reference wavelength is available, read only at 450nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 450 nm against 630 nm as reference.

◆ Assay Procedure

1. Dilute standard: Prepare sixs test tube ,make number successively,add Standard diluent 100ul to ervery test tube,add Original density Standard 100ul to the first test tube, Gently mix;then take out 100ul from the first test tube and add to the second test tube, Gently mix; then take out 100ul from the second test tube and add to the third test tube, Gently mix; then take out 100ul from the third test tube and add to the forth test tube, Gently mix; then take out 100ul from the forth test tube and add to the fifth test tube, Gently mix; take out 100ul from the fifth test tube and Discard,make the sixth test tube as standard 0. the density Standard of every test tube is :120 ng/ml,60 ng/ml,30 ng/ml,15 ng/ml, 7.5ng/ml,0 ng/ml .

   set Standard wells on the Microelisa Stripplate , add different concentrations of standard 50 ul successively .
2. Add samples: Set blank wells separately (blank comparison wells don't add samples ,Biotinylated anti –CysC -antibody and HRP-Conjugate reagent), testing sample wells. add Sample 40μl to testing sample well, then add Biotinylated anti –CysC -antibody 10μl , don't touch the well wall as far as possible, and Gently mix.
3. Incubate: After closing plate with Closure plate membrane ,incubate for 30 min at 37℃.
4. Configurate liquid: 30-fold Wash Buffer Concentrate diluted 30-fold with distilled water and reserve.
5. washing：Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.
6. add enzyme：Add HRP-Conjugate Reagent 50μl to each well, except the blank well.
7. incubate：Operation with 3.
8. washing：Operation with 5.
9. color： add Chromogen Solution A 50μl and Chromogen Solution B 50μl to each well. Gently mix, incubate for 15 min at 37℃.
10. Stop the reaction： Add Stop Solution50μl to each well, Stop the reaction(the blue color change to yellow color Immediately).
11. assay： take blank well as zero , measure the optical densit (OD) at 450 nm after Adding

Stop Solution and within 15min.

CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of standards, controls and patient samples.
- Construct a standard curve by plotting the mean absorbance obtained from each standard against its.
- Concentration with absorbance value on the vertical(Y) axis and concentration on the horizontal (X) axis.
- Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
- Automated method: The results in the IFU have been calculated automatically using a 4 PL.
- (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. Other data.
- Reduction functions may give slightly different results.
- The concentration of the samples can be read directly from this standard curve. Samples with.
- Concentrations higher than that of the highest standard have to be further diluted. For the calculation of.
- The concentrations this dilution factor has to be taken into account.

REFERENCES

REF : Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat

LOT : Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.:

:No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests:

: Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. /

Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la

luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari.

: Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. /Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso.

: Store at: / Lagern bei: / Stocker à: / Almacene a :/ Armazenar a :/ Conservare a:

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人半胱氨酸蛋白酶抑制蛋白C（Cys C）酶联免疫分析(ELISA)试剂盒使用说明书

货号：HM10642

- 本试剂盒用于体外定量检测人血清、血浆、组织、细胞上清及相关液体样本中半胱氨酸蛋白酶抑制蛋白C（Cys C）的含量。
- 有效期：6个月
- 保存条件：2-8℃

使用目的

本试剂盒用于体外定量检测人血清、血浆、组织、细胞上清及相关液体样本中半胱氨酸蛋白酶抑制蛋白C（Cys C））的含量。

实验原理

本试剂盒应用双抗体夹心法测定标本中人半胱氨酸蛋白酶抑制蛋白C（Cys C）水平。用纯化的人半胱氨酸蛋白酶抑制蛋白C（Cys C）抗体包被微孔板，制成固相抗体，往包被单抗的微孔中依次加入半胱氨酸蛋白酶抑制蛋白C（Cys C），再与HRP标记的半胱氨酸蛋白酶抑制蛋白C（Cys C）抗体结合，形成抗体-抗原-酶标抗体复合物，经过彻底洗涤后加底物TMB显色。TMB在HRP酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的半胱氨酸蛋白酶抑制蛋白C（Cys C）呈正相关。用酶标仪在450nm波长下测定吸光度（OD值），通过标准曲线计算样品中人半胱氨酸蛋白酶抑制蛋白C（Cys C）浓度。

注意事项

- 本试剂盒仅用于科研，不得用于医学诊断。
- 使用试剂盒前，请仔细阅读说明书，以试剂盒内的说明书为准，请务必理解说明书内容。
- 酶标包被板属于可拆型，开封后如未用完，板条应装入铝箔袋密封并2-8°C保存。
- 加样样本和试剂应尽快完成。
- 避免实验过程中酶标板干燥；清洗后尽快加试剂。
- 实验开始，所有试剂应平衡到室温（21-26°C）后方可进行。温度会影响吸光度值，但不影响样本值。
- 切忌用口加样，以免试剂和样本粘到皮肤和粘膜上。
- 不要在处理样本或试剂的区域抽烟、吃东西、喝酒或化妆。
- 操作时带一次性手套，微生物的污染会影响实验结果的正确性。
- 操作应按照国家规定的安全条列进行。
- 过期的试剂盒切勿再使用。
- TMB 底物对皮肤有刺激性，不慎入眼，立即用大量水冲洗，皮肤上不慎接触到也应立即用肥皂，并用大量的水冲洗。试验中被污染的物品在下次使用前应尽快清洗。

试剂盒组成

1.说明书	**1份**
2.封板膜	**2张**
3.酶标包被板	**12孔×8条**
4.标准　240 ng/ml	**0.6ml×1 瓶**
5.标准品稀释液	**2ml×1 瓶**
6.生物素标记的抗Cys C抗体	**1.5ml×1瓶**
7.显色剂A液	**6ml×1瓶**

8.显色剂B液 **6ml×1瓶**
9.终止液 **6ml×1瓶**
10.酶标试剂 **6ml×1瓶**
11. 30倍浓缩洗涤液 **20ml×1瓶**

需要而未提供的试剂和器材

- 吸光度值在450nm波长下检测的酶标仪。
- 1-2ml的加样器。
- 100 ml和1L的量筒。
- 吸水纸。
- 37° C 恒温箱。
- 蒸馏水或去离子水。
- 数据分析和绘图软件，图纸。
- 标准和样品稀释的试管。

储存条件

- ◆ 有效期内的试剂如开封后未用完，请2-8° C 保存。
- ◆ 过期的试剂请不要用，打开后的试剂2-8° C 保存。
- ◆ 酶标包被板必须2-8° C 保存，如有未用完的酶标包被板，打开的铝箔袋须密封并2-8° C 保存。
- ◆ 开封后的试剂盒2-8°C只能保存8周。

试剂准备

试剂盒从冷藏环境中取出应在室温平衡后方可使用。

样本处理及要求

1. **血清**-用采血管收集血液，室温血液自然凝固30分钟，1000 xg离心15分钟左右，收集上清，尽早进行实验，或者分装后-20°C 或 -80°C 保存。
2. **血浆**-收集好的血浆根据要求选择EDTA或者柠檬酸钠作为抗凝剂，2-8°C 1000 xg离心15分钟左右，30分钟内收集好，-20°C 或 -80°C 保存，避免反复冻融。
3. **细胞上清及相关液体**-收集好的样本离心后应尽快实验，或者分装后-20°C 或 -80°C 保存，避免反复冻融。

操作步骤

◆ 注意事项

- 所有试剂和样本使用前必须在室温下平衡，混匀试剂时不应起泡沫。
- 一旦实验开始，各操作步骤都应尽快完成。
- 避免重复使用手中的吸头和试管，以免交叉污染。
- 一般情况，酶的反应与时间和温度成正比。

◆ 操作步骤

1. 标准品的稀释：准备小试管 6 只，依次编好号码，先在各小试管中加入标准品稀释液 100ul，然后取原浓度标准品 100ul 加入一只已编好号的试管中，充分混匀;再在该试管中取 100ul 加入第二支试管中，充分混匀；再在该试管中取 100ul 加入第三只试管中，充分混匀；再在该试管中取 100ul 加入第四只试管中，充分混匀；再在该试管中取 100ul 加入第五只试管中，充分混匀；然后在该试管中取 100ul，弃掉。第六只试管作为 0 号标准品。稀释后各管浓度分别是：120 ng/ml,60 ng/ml,30 ng/ml,15 ng/ml, 7.5ng/ml,0 ng/ml。

   在酶标包被板上设标准品孔，依次加入不同浓度的标准品 50ul（建议每个浓度做 2 个平行孔）。
2. 加样：分别设空白孔（空白对照孔不加样品、酶标试剂及生物素标记的抗 Cys C 抗体，其余各步操作相同）、待测样品孔。在酶标包被板上待测样品孔中先加样品 40μl，然后再加生物素标记的抗 Cys C 抗体 10μl。加样将样品加于酶标板孔底部，尽量不触及孔壁，轻轻晃动混匀。
3. 温育：用封板膜封板后置 37℃温育 30 分钟。
4. 配液：将 30 倍浓缩洗涤液用蒸馏水 30 倍稀释后备用。
5. 洗涤：小心揭掉封板膜，弃去液体，甩干，每孔加满洗涤液，静置 30 秒后弃去，如此重复 5 次，拍干。
6. 加酶：每孔加入酶标试剂 50μl，空白孔除外。
7. 温育：操作同 3。
8. 洗涤：操作同 5。
9. 显色：每孔先加入显色剂 A50μl，再加入显色剂 B50μl，轻轻震荡混匀，37℃避光显色 15 分钟.
10. 终止：每孔加终止液 50μl，终止反应（此时蓝色立转黄色）。
11. 测定：以空白空调零，450nm 波长依序测量各孔的吸光度（OD 值）。 测定应在加终止液后 15 分钟以内进行

实验结果计算

以标准物的活性为横坐标，OD值为纵坐标,在坐标纸上绘出标准曲线，根据样品的OD值由标准曲线查出相应的活性；再乘以稀释倍数；或用标准物的活性与OD值计算出标准曲线的直线回归方程式，将样品的OD值代入方程式，计算出样品活性，再乘以稀释倍数，即为样品的实际活性。