优品生物
UpingBio

(For scientific research use only, not for clinical diagnosis!)

Human Telomerase Reverse Transcriptase (TERT) Quantitative Detection Kit (ELISA) Instructions for Use Specification: 96T/48T Catalog Number: SYP-H0204

Purpose: Used to detect human telomerase reversal in serum, plasma, cell culture supernatant and other samples

The concentration of transcriptase (TERT).

Please read the instructions carefully before use. If you have any questions, please contact us via:

Official hotline: 400-999-8863

Technical phone number: 18358180525

Email: UpingBio@163.com

Company website: www.upingbio.com **For specific shelf life, please refer to the outer packaging label of the kit. Please use the kit within the shelf life. When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.**

[Kit performance]

Physical properties: Each liquid component is clear and transparent, with no sediment or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

Calibration curve linearity: The correlation coefficient r value of the calibrator dose-response curve is greater than or equal to 0.9900. Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%. Sensitivity: The lowest detectable dose is less than 0.078 ng/ml.

Recovery rate: The recovery rate is between 85%-115%.

Sensitivity: This kit recognizes natural human telomerase reverse transcriptase (TERT) and has no crossover with structural analogs.

Stability: Stored at 2℃-8℃, validity period is 6 months.

Detection range: 0.312 ng/ml – 10 ng/ml.

【Experimental principle】

本试剂盒采用双抗体夹心法酶联免疫吸附试验（ELISA）。在预包被抗 人端粒酶逆转录酶(TERT)抗体（固相抗体）的微孔酶标板中，加入人端粒 酶逆转录酶(TERT)校准品和待测样本，再加入生物素标记抗体，经过温育 与充分洗涤后，再加入 HRP 偶联的亲和素，经过温育与充分洗涤，去除 未结合的组分，在微孔板固相表面形成固相抗体-抗原-生物素标记抗体-亲和素酶的夹心复合物。加 TMB 显色液，产生蓝色产物，在终止液作用 下，最终转化为黄色，在酶标仪 450nm 波长上测定吸光度（OD 值），吸 光度（OD 值）与待测样品中人端粒酶逆转录酶(TERT)的浓度正相关。拟 合校准品曲线，可以计算出样本中人端粒酶逆转录酶(TERT)的浓度。

【试剂盒组分与保存】

组分		数量	主要成分
校准品	High Standard	2 vial	校准品冻干粉
校准品复溶液	Reconstitution	2 vial	PBS
校准品＆样本稀释液	Standard & Sample Diluent	25mL	PBSTN
包被微孔板	Microelisa Stripplate	96T/48T	预包被固相抗体
生物素抗体	Bio-Antibody	10mL	生物素抗体
HRP 标记亲和素	HRP-Conjugate	10mL	HRP 标记亲和素
TMB 显色液	TMB	10mL	TMB
终止液	Stop Solution	6mL	酸性溶液
20×浓缩洗涤液	20X Wash Solution	25mL	0.05%Tween20
说明书	说明书	1 份	--
自封袋	自封袋	1 个	--
不干胶	不干胶	4 片	--

注意：1、使用前请检查试剂盒中试剂的标签和数量与表格是否一致。

2、试剂盒 2-8℃保存，不得使用过期试剂盒。

3、包被微孔板单次未使用完，要谨记密封放到 2-8℃保存。

4、复溶后的校准品仅限当天使用。

5、如果试剂盒的组份需要再次使用，请确保上一次使用之后没有被污染。

Prepare your own test equipment required for the test (not provided, but can assist in purchasing) 1.

Standard specification microplate reader.

2. Automatic plate washing machine.

3. Oscillator.

4. A series of adjustable pipettes and tips. When testing a large number of samples at one time, it is best to use a multi-channel pipette.

[Kit limitations]

1. For scientific research use only and not for clinical diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.

3. Do not mix with kits or components from other manufacturers.

4. Use the sample diluent provided with the kit.

5. If the sample value is higher than the highest calibrator concentration value, please dilute the sample appropriately and then re-measure. 6. Human anti-mouse

and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.

7. The test results obtained by other methods are not directly comparable to the test results of this kit.

【Precautions】

1. This kit is for in vitro research only and not for clinical diagnosis.

2. Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

3. Incubate strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

4. Incorrect plate washing may lead to inaccurate results. Be sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

5. Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.

6. The substrate chromogenic solution should be colorless or very light in color.

7. Avoid cross-contamination of reagents and specimens to avoid erroneous results.

8. Avoid direct exposure to strong light during storage and incubation.

9. After balancing to room temperature, open the sealed bag to prevent water droplets from condensing on the cold slats.

10. Any reaction reagents must not come into contact with bleaching solvents or strong gases emitted by bleaching solvents. Any bleaching ingredients will destroy the biological activity of the reagents in the kit.

11. The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of 450±10nm.

Optical density ranges from 0-3.5. It is recommended to preheat 15 minutes in advance before use.

12. Do not mix or replace the reagents in this kit with reagents from other batch numbers or other sources. 13. The EP tubes and suction tips used in the test are single-use and are strictly prohibited from mixing.

14. Do not use expired reagents.

【Sample preparation and storage】

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide shall not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant: Centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum: Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma: Anticoagulate with heparin, citrate or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood drawing. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis medium: For adherent cells, remove the culture medium and replace with PBS, physiological saline or blood-free

Wash with culture medium. Add an appropriate amount of lysis solution and pipet several times to ensure full contact between the lysis solution and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them once with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, pipet with a pipette to disperse the cells, and flick with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Urine: Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

【Reagent preparation】

1. Before use, all components must be rewarmed for at least 120 minutes to ensure full rewarming to room temperature.

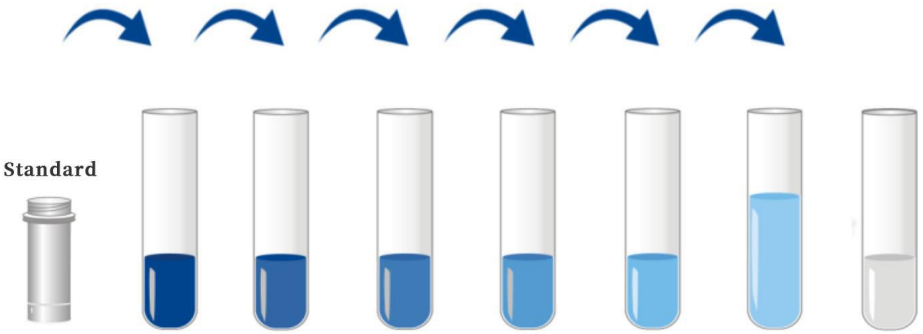
2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating

in a water bath will completely dissolve the crystals. Concentrated detergent and distilled water, dilute 1:20, that is, 1 part of concentrated detergent, add 19 parts of distilled water.

[Calibration dilution method]

校准品重溶步骤：用校准品复溶液重溶校准品，将一瓶校准品复溶液中的液体，全部加入到一瓶的校准品冻干粉中，轻柔地涡旋震荡，确保充分混匀，重溶后校准品的母液浓度为 20 ng/ml ,稀释前充分混匀。

校准品母液稀释步骤：稀释前校准品工作液静置 1-2 分钟，用校准品＆样本通用稀释液将校准品母液进行倍比稀释，倍比稀释方法：取 7 支 EP 管，每管中加入 500 µL 校准品＆样品稀释液，从 20 ng/ml 的校准品母液中吸取 500 µL 到第一支 EP 管中混匀配成 10 ng/ml 的校准品工作液，按此步骤往后依次吸取混匀。如下页图示。



20 ng/ml

校准品建议稀释浓度：建议配制成以下浓度：10、5、2.5、1.25、0.625、

0.312、0 ng/ml，并作为拟合标曲的校准品浓度值。

提示：最后一管直接加入校准品＆样本通用稀释液作为 0 值，不需要再从倒数第二管中吸取液体。倍比稀释的校准品工作液需要现配现用。

【操作程序】

推荐样本稀释方案：建议老师先做预实验摸索样本最佳稀释倍数，然后再做正式实验。

所有试剂和组分都先恢复到室温，校准品、质控品和样品，建议做复孔。

1、按前面说明书描述的方法，配制好试剂盒各种组分的工作液。

2、从铝箔袋中取出所需板条，剩余的板条用自封袋密封放回冰箱。

3、设置校准品孔、样本稀释液孔、空白孔和样本孔，校准品孔各加不 同浓度的校准品 50μL，样本稀释液孔加样本稀释液 50μL，空白孔不加，样本孔加待测样本 50μL。除空白孔外，每孔加入生物素抗体 100uL，用 封板膜盖住反应板，37℃水浴锅或恒温箱避光温育 60min。

4、揭开封板膜，弃去液体，吸水纸上拍干，每孔加满洗涤液，静置

1 minute, shake off the washing liquid, pat dry on absorbent paper, repeat this 5 times. If you use an automatic plate washer, please wash the plate according to the operating procedures of the plate washer. Adding a soaking program for 30 seconds can improve the detection accuracy. After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

5. Except for the blank wells, add 100uL of HRP-labeled avidin to each well, cover the reaction plate with a sealing film, and incubate in a 37℃ water bath or incubator in the dark for 20 minutes.

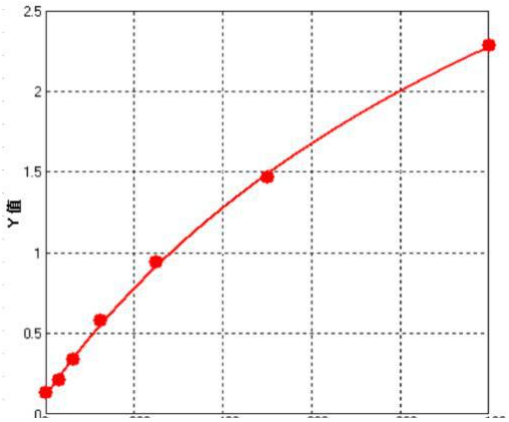
6. Repeat step 4.

7. Add 100μL of TMB chromogenic solution to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator in the dark for 15 minutes.

8. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well on a 450 nm wavelength microplate reader.

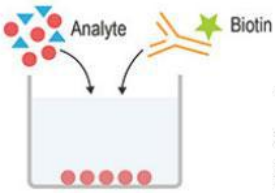
【Result calculation】

1. Use the concentration of the calibrator as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software] 2. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.

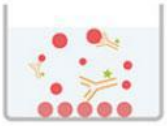


(Schematic diagram, for reference only)

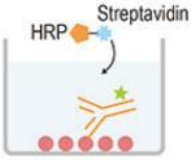
[Operation Summary]



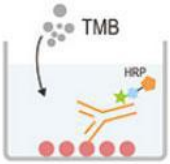
1、反应板孔中加入50uL校准品工作液或样本后,立即每孔加入100uL生物素化抗体工作液，37℃孵育60分钟。



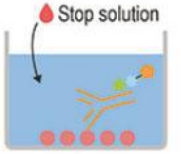
2、弃掉板内液体，洗板5次。



3、每孔加入100uL HRP酶结合物工作液37℃孵育20分钟，弃掉板内液体，洗板5次。



4、每孔加入100uL TMB显色液，37℃孵育 15分钟。



5、每孔加入 50uL 终止液。

6、立即在 450nm波长下读数，处理数据。

【problem analysis】

Problem Description	Possible Causes	Corresponding
Negative and positive control results are unstable	Incorrect liquid	Check pipettes and tips
	Equilibration time is too	Ensure sufficient
	Incomplete washing	Ensure the washing time and number of washes
Very weak or colorless	Incubation time too short	Ensure adequate
	Experimental	Use recommended
	Insufficient reagent	Check the liquid aspiration and addition
	Incorrect dilution	
	Enzyme label inactivation or substrate	Mix enzyme conjugate and substrate and check
Reading value is low	Microplate reader settings are incorrect	在酶标仪上检查波长及滤 光片设置
		提前打开酶标仪预热
变异系数大	加液不正确	检查加液情况
背景值高	检测抗体的工作浓度过	使用推荐的稀释倍数
	酶标板洗涤不完全	保证每步清洗完全；如果用 自动洗板机，请检查所有 的出口是否有堵塞；是否使 用
	洗液有污染	配制新的洗液
灵敏度低	ELISA 试剂盒保存不当	按说明书要求保存相关试 剂
	读数前未终止	OD 读数前应在每孔中加 入终止液

若实验效果不好，请及时对显色结果拍照，保存实验数据，保留所用板条及未使用试剂，然后联系我公司技术支持为您解决问题。

【声明】

1、限于现有条件及科学技术水平，尚不能对所有原料进行全面的鉴定 分析，本产品可能存在一定的质量技术风险。

2、本试剂盒在研发过程中去除/降低了生物学样本中的一些内源性干扰因素，并非所有可能影响的因素均已去除。

3、最终的实验结果与试剂的有效性、实验者的相关操作以及当时的实 验环境等因素密切相关，本公司只对试剂盒本身负责，不对因使用试剂盒 所造成的样本消耗负责，请使用者使用前充分考虑到样本可能的使用量，预留充足的样本。

4、为了达到好的实验结果，请只使用本公司试剂盒内提供的试剂，不 要混用其他制造商的产品，严格按照说明书操作。

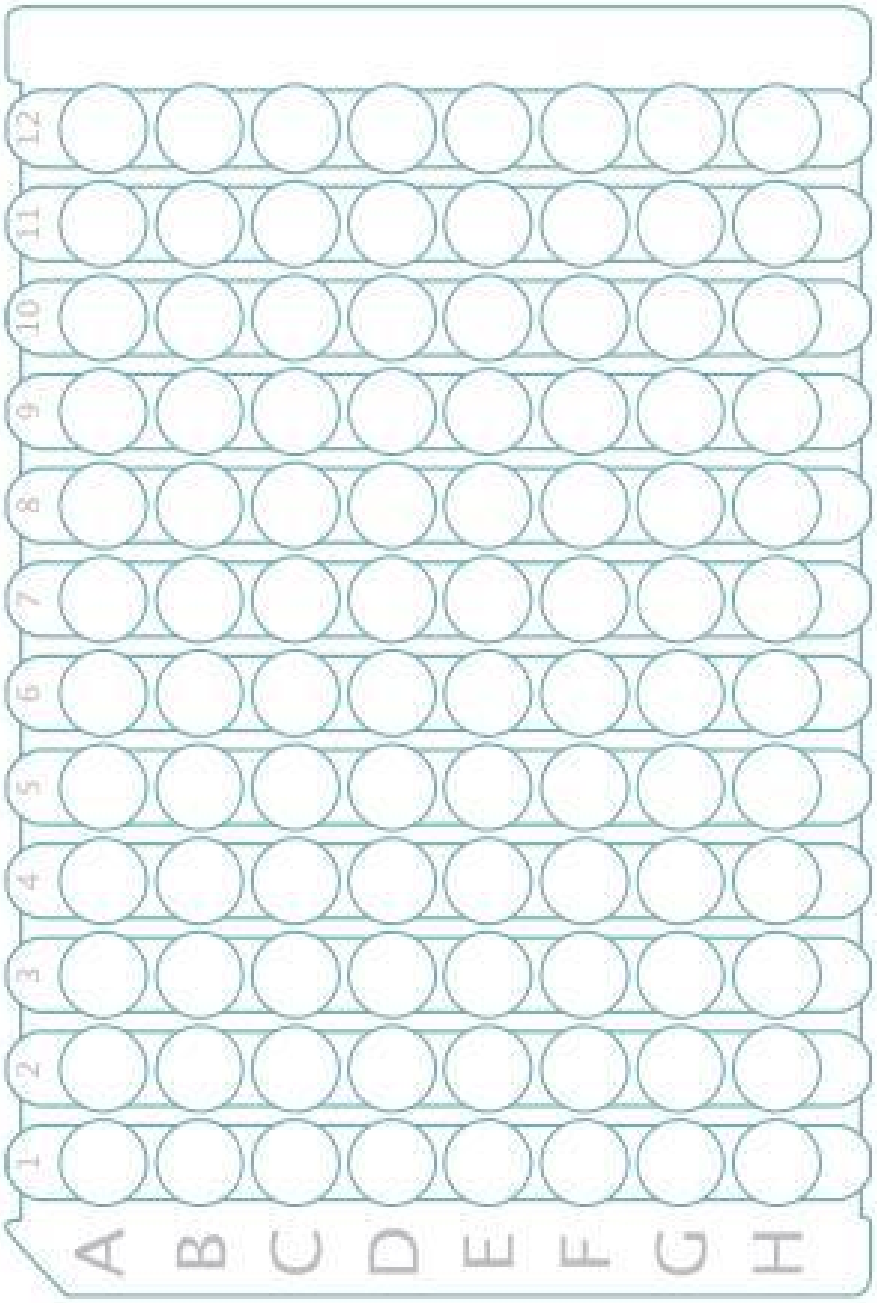
5、由于操作过程中试剂制备以及酶标仪参数设置不正确，可能导致结 果异常，实验前请仔细阅读说明书并调整好仪器。

6、即使是相同人员操作也可能在两次独立实验中得到不同的结果，为 保证结果的重现性，需要控制实验过程中每一步的操作。

7、试剂盒发货前会经过严格的质检，然而，因为运输条件、实验设备 差异等等因素影响，用户检测结果可能跟出厂数据不一致。

8、本试剂盒未与其他厂家同类试剂盒或不同方法检测同一目的物的产 品进行对比，所以不排除检测结果不一致的情况。

9、试剂盒仅供研究使用，如将其用于临床诊断或任何其他用途，我公 司将不对因此产生的问题负责，亦不承担任何法律责任。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【实验心得】

【实验心得】