**Human TPIgGAb ELISA Kit**

**For the qualitative in vitro determination of Human Treponema pallidum IgG antibody**

**concentrations in**



**serum - plasma - tissue homogenates - other biological fluids**

FOR LABORATORY RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

***This package insert must be read in its entirety before using this product.***

**ELISA**

**ENZYME LINKED IMMUNOSORBENT ASSAY**

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**INTENDED USE AND TEST PRINCIPLE**

This TPIgGAb ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of TPIgGAb in the sample, this TPIgGAb ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus TPIgGAb concentration. The concentration of TPIgGAb in the samples is then determined by comparing the O.D. of the samples to the standard curve.

**SAMPLE COLLECTION AND STORAGES**

**Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature orovernight at 4℃ before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15minutes at 1000×g at 2-8℃ within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

**Tissue homogenates** - For general information, hemolysis blood may affect the result, so youshould rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

**Cell culture supernates and other biological fluids -** Centrifuge samples for 20 minutes at1000×g. Remove particulates and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

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**Note:** The samples shoule be centrifugated dequately and no hemolysis or granule wasallowed.

**MATERIALS REQUIRED BUT NOT SUPPLIED**

1. 37 ℃ incubator
2. Standard microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes, disposable pipette tips and Absorbent paper
4. Distilled or deionized water

**REAGENTS PROVIDED**

*All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.*

|  |  |  |
| --- | --- | --- |
| Name | 96 determinations | 48 determinations |
|  |  |  |
| MICROTITER PLATE | 96 strips | 48 strips |
|  |  |  |
| Negative control | 0.3ml/vial | 0.3ml/vial |
|  |  |  |
| Positive control | 0.3ml/vial | 0.3ml/vial |
|  |  |  |
| Sample diluent | 6.0ml | 3.0ml |
|  |  |  |
| ENZYME CONJUGATE | 10.0ml | 5.0ml |
|  |  |  |
| WASH SOLUTION | 25ml | 15ml |
|  |  |  |
| SUBSTRATE A | 6.0ml | 3.0ml |
|  |  |  |
| SUBSTRATE B | 6.0ml | 3.0ml |
|  |  |  |
| STOP SOLUTION | 6.0ml | 3.0ml |
|  |  |  |
| Closure plate membrane | 2 | 2 |
|  |  |  |
| User manual | 1 | 1 |
|  |  |  |
| Sealed bags | 1 | 1 |
|  |  |  |

**PRECAUTIONS**

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use

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water baths to thaw samples or reagents.

1. Do not use kit components beyond their expiration date.
2. Use only deionized or distilled water to dilute reagents.
3. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
4. Use fresh disposable pipette tips for each transfer to avoid contamination.
5. Do not mix acid and sodium hypochlorite solutions.
6. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
7. All samples should be disposed of in a manner that will inactivate viruses.
8. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
9. Substrate Solution is easily contaminated. If bluish prior to use, do not use.
10. Substrate B contain 20% acetone, keep this reagent away from sources of heat or flame.

13. Remove all kit reagents from refrigerator and allow them to reach room temperature ( 20-25°C).

**REAGENT PREPARATION AND STORAGE**

**Wash Solution (1X) -** Dilute 1 volume of Wash solution (20X) with 19 volumes of deionized ordistilled water. Wash Solution is stable for 1 month at 2-8°C.

**ASSAY PROCEDURE**

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microtiter plate.
2. Add 50μl of Negative control，Positive control and test sample to the appropriate wells. Blank well doesn’t add anyting.
3. Add 100μl of Enzymeconjugate to standard wells and sample wells except the blank well,

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cover with an adhesive strip and incubate for 60 minutes at 37°C.

4 . Wash the Microtiter Plate 4 times.

**Manual Washing** - Remove incubation mixture by aspirating contents of the plate into a sink orproper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

**Automated Washing** - Aspirate all wells, then wash plates four times using Wash Buffer (1X).Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350μL/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

1. Add Substrate A 50μl and Substrate B 50μl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**
2. Add 50μl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
3. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

**DETERMINE THE RESULT**

1. Test validity: the average of Positive control well≥0.8; the average of Negative control well

≤0.2.

1. Calculate Critical (CUT OFF): Critical= the average of Negative control well + 0.25.
2. Negative Result: sample OD< Calculate Critical (CUT OFF) is Negative.
3. Positive Result: sample OD≥ Calculate Critical (CUT OFF) is Positive.

**CALCULATION OF RESULTS**

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard

concentrations on the vertical (X) axis versus the corresponding concentration on the horizontal

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(Y) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. Values are subtracted by the mean value of the balnk well before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
3. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.
4. Intra-assay CV(%) and Inter-assay CV（％）are less than 15%.
5. Cross-reactivity: No significant cross-reactivity or interference was observed.
6. Storage: 2-8℃ (Use frequently); six months (-20℃)。

**FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS! PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

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**人梅毒螺旋体Igg抗体（TPIgGAb）试剂盒（ELISA）**

使用说明书

1. **本试剂盒用于体外定性检测血清、血浆、组织匀浆及相关液体样本中人梅毒螺旋体Igg抗体（TPIgGAb）。**
2. **有效期：6个月**
3. **保存条件：2-8℃**

**实验原理**

试剂盒采用间接法酶联免疫吸附试验（ELISA）。往预先包被人梅毒螺旋体Igg抗体（TPIgGAb）捕获抗原的包被微孔中，依次加入标本、阴性和阳性对照，再加入HRP标记的检测抗体，经过温育并彻底洗涤。用底物TMB显色，TMB在过氧化物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人梅毒螺旋体Igg抗体（TPIgGAb）呈正相关。用酶标仪在450nm 波长下测定吸光度（OD 值），判定阴阳性。

**样本处理及要求**

1. **血清：**将收集于血清分离管的全血标本在室温放置2小时或4℃过夜，然后1000×g离心20

分钟，取上清即可，或将上清置于-20℃或-80℃保存，但应避免反复冻融。

1. **血浆：**用EDTA或肝素作为抗凝剂采集标本，并将标本在采集后的30分钟内于2-8℃ 1000

×g离心15分钟，取上清即可检测，或将上清置于-20℃或-80℃保存，但应避免反复冻融。

1. **组织匀浆：**用预冷的PBS (0.01M, pH=7.4)冲洗组织，去除残留血液（匀浆中裂解的红细胞会影响测量结果），称重后将组织剪碎。将剪碎的组织与对应体积的PBS（一般按1:9的重量体积比，比如1g的组织样品对应9mL的PBS，具体体积可根据实验需要适当调整，并做好记录。

推荐在PBS中加入蛋白酶抑制剂）加入玻璃匀浆器中，于冰上充分研磨。为了进一步裂解组织细胞，可以对匀浆液进行超声破碎，或反复冻融。最后将匀浆液于5000×g离心5~10分钟，

取上清检测。

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1. **细胞培养物上清或其它生物标本：**请1000×g离心20分钟，取上清即可检测，或将上清置于-20℃或-80℃保存，但应避免反复冻融。

注：标本溶血会影响最后检测结果，因此溶血标本不宜进行此项检测。

**需要而未提供的试剂和器材**

1. 酶标仪（450nm）
2. 高精度加样器及枪头：0.5-10uL、2-20uL、20-200uL、200-1000uL
3. 37℃恒温箱
4. 蒸馏水或去离子水

**试剂盒组成**

|  |  |  |  |
| --- | --- | --- | --- |
| **名称** | **96 孔配置** | **48 孔配置** | **备注** |
|  |  |  |  |
| 微孔酶标板 | 96 孔 | 48 孔 | 无 |
|  |  |  |  |
| 阴性对照 | 0.3mL | 0.3mL | 无 |
|  |  |  |  |
| 阳性对照 | 0.3mL | 0.3mL | 无 |
|  |  |  |  |
| 样本稀释液 | 6mL | 3mL | 无 |
|  |  |  |  |
| 检测抗体-HRP | 10mL | 5mL | 无 |
|  |  |  |  |
| 20×洗涤缓冲液 | 25mL | 15mL | 按说明书进行稀释 |
|  |  |  |  |
| 底物 A | 6mL | 3mL | 无 |
|  |  |  |  |
| 底物 B | 6mL | 3mL | 无 |
|  |  |  |  |
| 终止液 | 6mL | 3mL | 无 |
|  |  |  |  |
| 封板膜 | 2 张 | 2 张 | 无 |
|  |  |  |  |
| 说明书 | 1 份 | 1 份 | 无 |
|  |  |  |  |
| 自封袋 | 1 个 | 1 个 | 无 |
|  |  |  |  |

**注意事项**

1. 严格按照规定的时间和温度进行温育以保证准确结果。所有试剂都必须在使用前达到室温 20-25℃。使用后立即冷藏保存试剂。
2. 洗板不正确可以导致不准确的结果。在加入底物前确保尽量吸干孔内液体。温育过程中不要让微孔干燥掉。
3. 消除板底残留的液体和手指印，否则影响 OD 值。
4. 底物显色液应呈无色或很浅的颜色，已经变蓝的底物液不能使用。
5. 避免试剂和标本的交叉污染以免造成错误结果。
6. 在储存和温育时避免强光直接照射。
7. 平衡至室温后再打开密封袋以防水滴凝聚在冷板条上。
8. 任何反应试剂不能接触漂白溶剂或漂白溶剂所散发的强烈气体。任何漂白成分都会破坏

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试剂盒中反应试剂的生物活性。

1. 不能使用过期产品。
2. 如果可能传播疾病，所有的样品都应管理好，按照规定的程序处理样品和检测装置。

**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。

20×洗涤缓冲液的稀释：蒸馏水按1：20稀释，即1份20×洗涤缓冲液加19份蒸馏水。

**操作步骤**

1. 从室温平衡 20min 后的铝箔袋中取出所需板条，剩余板条用自封袋密封放回 4℃。
2. 设置阴性对照孔、阳性对照孔和样本孔，阴性、阳性对照孔各加 50μL 对照品，样本孔中加入待测样本 50μL，空白孔不加。
3. 除空白孔外，对照孔和样本孔中每孔加入辣根过氧化物酶（HRP）标记的检测抗体 100μL，用封板膜封住反应孔，37℃水浴锅或恒温箱温育 60min。
4. 弃去液体，吸水纸上拍干，每孔加满洗涤液（350μL），静置 1min，甩去洗涤液，吸水纸

上拍干，如此重复洗板 5 次（也可用洗板机洗板）。

1. 每孔加入底物 A、B 各 50μL，37℃避光孵育 15min。
2. 每孔加入终止液 50μL，15min 内，在 450nm 波长处测定各孔的 OD 值。

**实验结果计算**

1. 阴性对照OD值：小于0.2。
2. 阳性对照OD值：大于0.8。

3、阳性判断（Cut-Off值）：阴性对照OD值+0.25，样本OD值大于阈值，判定为阳性，反之，

为阴性。

4、重复性：板内变异系数小于15%。

5、储藏：2-8℃避光密封保存。

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