# Mouse anti-double stranded DNA antibody (IgG) ELISA Kit

**Catalog Number. CSB-E11194m**

**For the qualitative determination of mouse anti-double stranded DNA antibody (IgG) concentrations in serum.**

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

**If You Have Problems**

**Technical Service Contact information**


Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com


In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

## PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique.
The microtiter plate provided in this kit has been pre-coated with antigen. Samples are pipetted into the wells with anti-mouse IgG conjugated Horseradish Peroxidase (HRP). Any antibodies specific for the antigen present will bind to the pre-coated antigen. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of mouse anti-double stranded DNA antibody(IgG) bound in the initial step. The color development is stopped and the intensity of the color is measured.

## SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of mouse anti-double stranded DNA antibody(IgG). No significant cross-reactivity or interference between mouse anti-double stranded DNA antibody(IgG) and analogues was observed.

**Note:** Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between mouse anti-double stranded DNA antibody(IgG) and all the analogues, therefore, cross reaction may still exist.

## PRECISION

**Intra-assay Precision (Precision within an assay): CV%<15%**
Three samples of known concentration were tested twenty times on one plate to assess.

**Inter-assay Precision (Precision between assays): CV%<15%**
Three samples of known concentration were tested in twenty assays to assess.

## LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

| Reagents | Quantity |
|---|---|
| Coated assay plate | 1(96 wells) |
| Negative Control | 1 x 800 µl |
| Positive Control | 1 x 800 µl |
| HRP-conjugate | 1 x 10 ml |
| Sample Diluent | 1 x 50 ml |
| Wash Buffer (25 x concentrate) | 1 x 20 ml |
| Substrate A | 1 x 5 ml |
| Substrate B | 1 x 5 ml |
| Stop Solution | 1 x 5 ml |
| Adhesive Strip (For 96 wells) | 4 |
| Instruction manual | 1 |

## STORAGE

| | |
|---|---|
| Unopened kit | Store at 2 - 8°C. Do not use the kit beyond the expiration date. |
| Opened kit | May be stored for up to one month at 2 - 8° C. |

***Provided this is within the expiration date of the kit.**

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100ml and 500ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## SAMPLE COLLECTION AND STORAGE

**Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

## SAMPLE PREPARATION

Dilute the serum samples with Sample Diluent(1:1000) before test. The suggested 1000-fold dilution can be achieved by adding 5μl sample to 95μl of Sample Diluent first, then complete the 1000-fold dilution by adding 5μl of this solution to 245μl of Sample Diluent.

**Note:**

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 2 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

## REAGENT PREPARATION

**Note:**

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.**
- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

**Wash Buffer(1x)-** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

**ASSAY PROCEDURE**

**Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples be assayed in duplicate.**

1. Prepare all reagents, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution.
4. Set three **Negative Control** wells, two **Positive Control** wells.
5. Add 100µl of **Negative Control**, **Positive Control** or **diluted Sample** per well. Cover with the adhesive strip provided. Incubate for 30 minutes at 37°C.
6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (300µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100µl of **HRP-conjugate** to each well (not to Blank!). Cover the microtiter plate with the adhesive strip. Incubate for 30 minutes at 37°C.
8. Repeat the aspiration/wash process for five times as in step 6.
9. Add 50µl of **Substrate A** and 50µl **Substrate B** to each well. Incubate for 10 minutes at 37°C. **Protect from light.**
10. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
11. Take blank well as zero, determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

***Samples may require dilution. Please refer to Sample Preparation section.**

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

**CALCULATION OF RESULTS**

For calculation the valence of mouse anti-double stranded DNA antibody(IgG), compare the sample well with control.

Negative Control OD values must less than 0.15. Positive Control OD Values must more than 0.6.

If ODnegative＜0.1, calculate it as 0.1.

A cutoff value was defined as the average Negative Control value plus 0.1.

- While ODsample≥Cutoff Value: Positive
- While ODsample＜Cutoff Value: Negative

# 小鼠抗双链 DNA 抗体/天然 DNA 抗体(IgG)酶联免疫试剂盒
# 使用说明书

**【产品编号】**CSB-E11194m

**【预期应用】**ELISA 法评价小鼠血清中抗双链 DNA 抗体(IgG)效价。

**【产品性能指标】**

1、 精密度：批内差 CV%<15%，批间差 CV%<15%

2、 特异性：本试剂盒特异性检测小鼠抗双链 DNA 抗体(IgG)。

**【实验原理】**

用特异性抗原包被酶标板，制成固相载体。向微孔中先加入待测样品进行反应，然后再加入辣根过氧化物酶标记抗小鼠 IgG 进行反应，经过彻底洗涤后用底物显色。底物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小鼠抗双链 DNA 抗体(IgG)呈正相关。用酶标仪在 450nm 波长下测定吸光度（OD 值），与阴性对照孔判断样品中小鼠抗双链 DNA 抗体(IgG)效价。

**【试剂盒组成成分】**

| 组份 | 装量 |
|---|---|
| 酶标板 (Assay plate) | 96 孔 |
| 阴性对照(Negative Control) | 1 x 800 µl/瓶 |
| 阳性对照(Positive Control) | 1 x 800 µl/瓶 |
| 酶结合物 (HRP-conjugate) | 1 x 10 ml/瓶 |
| 样本稀释液 (Sample Diluent) | 1 x 50 ml/瓶 |
| 浓洗涤液 (Wash Buffer) | 1 x 20 ml/瓶 (25×) |
| 底物溶液 A (Substrate A) | 1 x 5 ml/瓶 |
| 底物溶液 B (Substrate B) | 1 x 5 ml/瓶 |
| 终止液 (Stop Solution) | 1 x 5 ml/瓶 |
| 板贴 | 4 |

**【存储条件及有效期】**

1. 未开封的试剂盒避光保存于2-8℃。有效期为六个月。请在试剂盒标注的有效日期内使用。
2. 酶标板打开后应置有干燥剂的铝箔袋中置于2-8℃密封防潮保存，2-8℃条件下最多可保存一个月。

**【所需试剂和器材】**

标准规格酶标仪；高速离心机；电热恒温培养箱；干净的试管和离心管；容量瓶；

系列可调节移液器及吸头；多通道移液器；蒸馏水 等

**【样本采集及保存】**

血清：全血标本请于室温放置 2 小时或 4℃过夜后于 1000g 离心 15 分钟，取上清即可立即检测；或进行分装，并将标本放于-20℃或-80℃保存，但应避免反复冻融。解冻后的样品应再次离心，然后检测。

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。

**【样本稀释】**

血清样本用样本稀释液进行 1:1000 倍稀释后进行检测，具体操作如下：取 5μl 样本加入到 95μl 的样本稀释液（1:20 稀释）中混匀。再从上述稀释液中取 5μl 加入到 245μl 样本稀释液（1:50 稀释）中混匀。二步完成后得到的即为 1:1000 倍稀释后的样本。

**【试剂配制】**

洗液工作液：洗液需提前配制，稀释前根据预先计算好的每次实验所需的总量配制。取出洗液浓缩液，浓洗涤液低温保存会有盐析出，稀释时可在水浴中加温助溶。浓洗涤液按1:25倍进行稀释。例如用量筒量取240ml去离子水，倒入烧杯或其他洁净容器中，再量取10ml浓洗涤液，均匀加入，搅拌混匀。

**【注意事项】**

1. 实验开始前，请提前配置好所有试剂。试剂或样品稀释时，均需混匀，混匀时尽量避免起泡。
2. 用户在初次使用试剂盒时，应将各种试剂管离心数分钟，以便试剂集中到管底。

**【操作步骤】**

1. 将各种试剂移至室温（18-25℃）平衡至少 30 分钟，按前述方法配制试剂，备用。
2. 将酶标板取出，设 3 个阴性对照孔，2 个阳性对照孔，1 个空白孔。
3. 每个检测孔直接加阴性对照、阳性对照或稀释后的待测标本各 100μl。
4. 充分混匀，贴上不干胶封片，置 37℃温育 30 分钟。
5. 弃去孔内液体，甩干，洗板 5 次。每次浸泡 2 分钟，300μl/孔，甩干。
6. 每孔加入酶结合物 100μl（空白对照孔除外），充分混匀，贴上不干胶封片，置 37℃温育 30 分钟。
7. 弃去孔内液体，甩干，洗板 5 次。每次浸泡 2 分钟，300μl/孔，甩干。
8. 每孔加底物溶液 A 和 B 各 50μl，振荡混匀后，37℃避光显色 10 分钟，每孔加终止液 50μl。
9. 空白孔调零，用酶标仪在 450nm 波长依序测量各孔的光密度（OD 值）。 在反应终止后 10 分钟内进行检测。

**【操作要点】**

1. 为保证检测结果的准确性，建议设双孔测定。
2. 加样时，请使用一次性的洁净吸头，避免交叉污染。加样时应尽量轻缓，避免起泡，将样品加于酶标板孔底部，切勿沿孔壁加样。
3. 为防止样品蒸发，温育过程中酶标板必须覆上板贴，实验过程中应避免酶标板处于干燥的状态。
4. 洗涤过程非常重要，不充分的洗涤易造成假阳性，在每次洗涤过程中，需要将孔内液体完全甩干，并在吸水纸上拍干，切勿将吸水纸直接放入反应孔中吸水，或用枪在孔中吸取液体。若用枪在孔中吸去液体，请勿触及板壁。
5. 一次加样时间最好控制在 10 分钟内，如标本数量多，推荐使用排枪加样。

**【洗板方法】**

手工洗板方法：吸去（不可触及板壁）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下用力拍几次；将推荐的洗涤缓冲液按 300μl/孔注入孔内，浸泡 2 分钟。根据需要，重复此过程数次。

自动洗板：如果有自动洗板机，应在熟练使用后再用到正式实验过程中。

**【数据处理】**

目测法：与阴性孔相比，有明显变色的为阳性，未有明显变色的为阴性。

读数法：判断值=0.1＋阴性孔平均 OD 值（阴性对照 OD 值小于 0.1 时，以 0.1 计算）

阴性对照孔的 OD 值≤0.15, 阳性对照孔的 OD 值≥0.6 时试验结果有效，否则应重新试验。

结果判定：样本 OD 值大于等于判断值为阳性，小于判断值为阴性。

**【说明】**

1. 本试剂盒仅供研究使用。
2. 中、英文说明书可能会有不一致之处，请以英文说明书为准。
3. 刚开启的酶标板孔中可能会含有少许水样物质，此为正常现象，不会对实验结果造成任何影响。
4. 不要用其它生产厂家的试剂替换试剂盒中的试剂。
5. 不同批号试剂不能混用。
6. 底物请避光保存。

Notes