Mouse anti-double stranded DNA, dsDNA ELISA Kit

Catalog No: E0288m
96 Tests
Operating instruction

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

Intended use
This immunoassay kit allows for the in vitro quantitative determination of mouse anti-double stranded DNA, dsDNA concentrations in cell culture supernates, serum, plasma and other biological fluids.

Introduction
Antibodies binding to DNA belong to the group of anti-nuclear antibodies (ANA) that have been observed in several autoimmune diseases. Antibodies reacting with native double-stranded (ds) DNA are regarded as being specific for systemic lupus erythematosus (SLE) and have been observed in approximately 50-80% of the patients. SLE is a chronic inflammatory autoimmune disease. It causes multiple organ damage and presents a variety of clinical and laboratory phenomena, particularly inflammation. Clinically, SLE is often accompanied by different autoantibodies, such as anti-double strand DNA Ab (anti-dsDNA Ab), anti-Smith Ab (anti-Sm Ab), anti-snRNP Ab, anti-Ro/La Ab (anti-SSa/SSb Ab) and ect.

Antibodies against dsDNA are found during active phases of SLE. The amount of the serum concentration is positively correlated with the severity of the disease. Thus, detection of these autoantibodies is important for the diagnosis and the clinical monitoring of SLE. Consequently it has been established as 1 of the 11 ACR-criteria for the diagnosis of SLE.

Most patients with SLE display IgG class antibodies against dsDNA. These autoantibodies are associated with lupus nephritis. Approximately 30% of the SLE patients develop IgA class anti-dsDNA antibodies, additionally. There have been suggestions that the presence of these IgA class anti-dsDNA antibodies may define a certain subset of SLE patients. Indeed studies demonstrated the association of this subclass with certain parameters of the disease activity, such as elevated erythrocyte sedimentation rate, or the consumption of complement component C3, as well as the clinical parameters of cutaneous vasculitis, acral necrosis and erythema. While no association was found for nephritis and arthritis.

IgM class anti-dsDNA antibodies were found in 52 % of the sera from patients with SLE. In contrast to IgG and IgA class autoantibodies, the subclass IgM antibodies do not correlate with disease activity. However, a highly significant negative correlation between IgM anti-dsDNA antibodies and lupus nephritis, including its laboratory parameters was
demonstrated. Therefore IgM class anti-dsDNA antibodies may indicate a subset of lupus patients being protected against the risk of developing nephritis.

**Materials and components**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay plate</td>
<td>1 × 20ml</td>
</tr>
<tr>
<td>Standard</td>
<td>2</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>1 × 20ml</td>
</tr>
<tr>
<td>Assay Diluent A</td>
<td>1 × 10ml</td>
</tr>
<tr>
<td>Assay Diluent B</td>
<td>1 × 10ml</td>
</tr>
<tr>
<td>Detection Reagent A</td>
<td>1 × 120μl</td>
</tr>
<tr>
<td>Detection Reagent B</td>
<td>1 × 120μl</td>
</tr>
<tr>
<td>Wash Buffer (25 x concentrate)</td>
<td>1 × 30ml</td>
</tr>
<tr>
<td>Substrate</td>
<td>1 × 10ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 × 10ml</td>
</tr>
<tr>
<td>Plate sealer for 96 wells</td>
<td>5</td>
</tr>
<tr>
<td>Instruction</td>
<td>1</td>
</tr>
</tbody>
</table>

**Other supplies required**

- Luminometer.
- Pipettes and pipette tips.
- EP tube
- Deionized or distilled water.

**Sample collection and storage**

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 × g at 2 - 8°C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

**Cell culture supernates 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.

**Note**: Serum, plasma, and cell culture supernatant samples to be used within 7 days may be stored at 2-8°C, otherwise samples must stored at -20°C (≤ 1 months) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.

**Limitations of the procedure**

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the
samples with the Assay Diluent and repeat the assay. Any variation in standard
diluent, operator, pipetting technique, washing technique, incubation time or
temperature, and kit age can cause variation in binding.

4. This assay is designed to eliminate interference by soluble receptors, ligands, binding
proteins, and other factors present in biological samples. Until all factors have been
tested in the Quantikine Immunoassay, the possibility of interference cannot be
excluded.

**Reagent preparation**

Bring all reagents to room temperature before use.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and
mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer
Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

**Standard** - Reconstitute the **Standard** with 1.0 mL of **Sample Diluent**. This reconstitution
produces a stock solution of 100 IU/ml. Allow the standard to sit for a minimum of 15
minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the
wells directly is not permitted). The undiluted standard serves as the high standard (100
IU/ml). The **Sample Diluent** serves as the zero standard (0 IU/ml).

![Image of dilution](image)

**Detection Reagent A and B** - Dilute to the working concentration using **Assay Diluent A**
and **B** (1:100), respectively.

**Assay procedure**

Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37°C
directly.). All the reagents should be mixed thoroughly by gently swirling before
pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and
remove extra strips from microtiter plate. Removed strips should be resealed and stored
at 4°C until the kits expiry date. Prepare all reagents, working standards and samples as
directed in the previous sections. Please predict the concentration before assaying. If
values for these are not within the range of the standard curve, users must determine the
optimal sample dilutions for their particular experiments.

1. Add 100 μl of **Standard**, Blank, or Sample per well. Cover with the Plate sealer.
   Incubate for 2 hours at 37°C.
2. Remove the liquid of each well, don't wash.
3. Add 100 μl of **Detection Reagent A** working solution to each well. Cover with the
   Plate sealer. Incubate for 1 hour at 37°C. **Detection Reagent A** working solution may
appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 μl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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.

5. Add 100 μl of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37°C.

6. Repeat the aspiration/wash as in step 4.

7. Add 90 μl of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37°C. Protect from light.

8. Add 50 μl of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Important Note:

1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.

2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10μl for once pipetting.

3. 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.

4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.

5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

7. Duplication of all standards and specimens, although not required, is recommended.

8. Substrate Solution is easily contaminated. Please protect it from light.

Specificity
This assay recognizes recombinant and natural mouse dsDNA. No significant cross-reactivity or interference was observed.

**Sensitivity**
The minimum detectable dose of mouse dsDNA is typically less than 0.78 IU/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

**Detection Range**
1.56 -100 IU/ml. The standard curve concentrations used for the ELISA's were 100 IU/ml, 50 IU/ml, 25 IU/ml, 12.5 IU/ml, 6.25 IU/ml, 3.12 IU/ml, 1.56 IU/ml.

**Calculation of results**
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the dsDNA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**Storage of test kits and instrumentation**
1. Unopened test kits should be stored referring to the package label for frequent use, and stored at -20°C for long time storage. The unused strips should be kept in a sealed bag and stored at 2-8°C in their pouch with the desiccant provided to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
2. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results.
3. Do not remove microtiter plate from the storage bag until needed.
4. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.
5. Use fresh disposable pipette tips for each transfer to avoid contamination.
6. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
7. Valid period: six months.

**Precaution**
The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.