Human anti-complement 1q antibody (anti-C1q-antibody)ELISA Kit

Catalog No. CSB-E09165h

(96 T)

- This immunoassay kit allows for the in vitro semi-quantitative determination of **human anti-C1q-antibody** concentrations in **serum**.
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

INTRODUCTION

Anti-C1q antibodies is a marker of lupus activity. Serum anti-C1q levels in patients with systemic lupus erythematosus (SLE), especially in patients with nephritis.Anti-C1q antibodies seem to correlate with active renal SLE.

One study showed that anti-C1q antibodies correlated with the proliferative forms of lupus nephritis and the rise in titres of anti-C1q preceded the development of clinical activity of disease by 6 months with a positive predictive value of 50 per cent.

Another study reported a correlation between the ongoing production of IgG anti-C1q and proliferative lupus nephritis. Anti-C1q antibody is not specific to SLE; in fact, it has been described in rheumatoid vasculitis, urticarial vasculitis, idiopathic membranoproliferative glomerulonephritis and IgA nephropathy.

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with specific antigen. Samples are then added to the appropriate microtiter plate wells and incubated. Then add Horseradish Peroxidase (HRP)-conjugated anti-human IgG and incubate again. Substrate solutions are added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. Calculate the valence of anti-C1q-antibody in the samples.

SPECIFICITY

This assay recognizes human anti-C1q-antibody. No significant cross-reactivity or interference was observed.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Sample Diluent	1 x 20 ml
HRP-conjugate	1 x 10 ml
Wash Buffer	1 x 20 ml (25×concentrate)
Positive Control	1 x 1 ml
Negative Control	1 x 1 ml
Substrate A	1 x 5 ml
Substrate B	1 x 5 ml
Stop Solution	1 x 5 ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

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 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARTION

Recommend to dilute the serum samples with Sample Diluent(1:101) before test. The suggested 101-fold dilution can be achieved by adding 2μl sample to 200μl of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and controls be assayed in duplicate.

1. Set one Blank well. Add 100μl of Sample Diluent to Blank well.
2. Add 100μl of **diluted sample**, **Positive Control** and **Negative Control** per well. Cover with the adhesive strip. Incubate for 30 minutes at 37° C.

3. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash Buffer** (200µl) 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.
4. Add 100µl of **HRP-conjugate** to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 30 minutes at 37° C.
5. Repeat the aspiration and wash five times as step 3.
6. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well. Incubate for 10 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
7. Add 50µl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Take Blank well as zero, determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each control and sample. For calculation the valence of human anti-C1q-antibody, compare the OD values of sample well with control (If the $OD_{negative}$ ＜0.1, calculate as 0.1) .

While OD_{sample}/ $OD_{negative}$≥2.1: Positive

While OD_{sample}/ $OD_{negative}$ ＜2.1: Negative

LIMITATIONS OF THE PROCEDURE

- 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.

TECHNICAL HINTS

- Centrifuge vials before opening to collect contents.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. 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 Substrate Solution.