DRG® C3d ELISA (EIA-2333)

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This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

Enzyme immunoassay for determination of circulating immune complex – C3d in human serum.

The C3d test system detects immune complexes containing both C3d and IgG.

TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with an antibody, directed towards an epitope of an antigen molecule. The antigen of the sample is incubated in the coated well with enzyme conjugated second antibody (E-Ab), directed towards a different region of the antigen molecule. After the substrate reaction the intensity of the developed color is proportional to the amount of the antigen. Results of samples can be determined directly using the standard curve.

WARNINGS AND PRECAUTIONS

1. For professional use only. In the United States, this kit is intended for Research Use Only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact DRG or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents of this kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2 – 8 °C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapter.
After opening the pouch for the coated microplates, store the unused strips in the resealed plastic bag with the desiccant included. Opened and unused strips are stable up to 30 d at 2-8°C.
The Standard must not be frozen. After opening it may be used up to 3 m.

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SPECIMEN COLLECTION AND STORAGE

Serum: The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples which appear turbid should be centrifuged before testing to remove any particulate material.			
Storage:	2-8°C	≤ -20°C (Aliquots)	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	72 h	> 72 h	

MATERIALS SUPPLIED

Quantity	Symbol	Component
12 x 8 wells	**MTP**	**Microtiter Plate** Break apart strips. Coated with monoclonal mouse-anti-C3d antibody.
1 x 15 mL	**ENZCONJ**	**Enzyme Conjugate** Pink colored. Ready to use. Contains human anti-human-IgG conjugated with Horseradish Peroxidase. Contains 0.05 % Proclin 300.
1 x 100 mL	**SAMPLEDIL**	**Sample Diluent** Blue colored. Ready to use. Contains 0.09 % Sodium azide.
3 x 2 x 1 mL	**CAL A-B**	**Standards A-B** Lyophilized Contains human antisera and 0.09 % Sodium azide. Exact concentrations see vial labels or QC Certificate.
1 x 450 µL	**CONTROL +**	**Positive Control** Contains human antisera and 0.09 % Sodium azide. Exact concentrations see QC Certificate.
1 x 450 µL	**CONTROL -**	**Negative Control** Contains human antisera and 0.09 % Sodium azide. Exact concentrations see QC Certificate.
1 x 50 mL	**WASHBUF**	**Wash Buffer Concentrate (20 x)** Contains Phosphate buffer with 0,02% Thimerosal.
1 x 15 mL	**TMB SUBS**	**TMB Substrate Solution** Ready to use. Contains Tetramethylbenzidine in buffer with stabilizers.
1 x 20 mL	**TMB STOP**	**Stop Solution** Ready to use. Contains 0.25 M H_2SO_4.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Distilled or deionised water
2. 1 L graduated cylinder
3. Wash bottle, automated or semi-automated microwell plate washing system
4. Rack for sample dilution
5. Vortex mixer
6. Pipettes 50, 100, 200 and 250 µL (Multipette Eppendorf or similar devices, less than 3% CV)
7. 8-Channel Micropipettor (for reagent delivery)
8. Reagent reservoirs for multichannel pipettes
9. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
10. Paper towels, pipette tips and timer

PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are ready prepared at the appropriate time. Allow all reagents and specimens to come to room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled evenly with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch with desiccant.

PRE-TEST SETUP INSTRUCTIONS

Dilution of Samples

Samples have to be diluted 1:5 with Sample Diluent, e.g. 50 µL + 200 µL. It is recommended to prepare fresh dilutions for each test run.

Dilution of Wash Buffer

Dilute **Wash Buffer (20x) 1:20** with **dist. or deionised water**, e.g. 50 mL up to 1 L. Mix well.	Storage: 2-8°C Stability: 1 w

Dilution of Control

Dilute **Control 1:5** with **Sample Diluent**, e.g. 50 µL with 200 µL. Mix carefully without foaming.	Prepare freshly directly before use and use only once.

Reconstitution of Standards

Pipette **0.15 mL bidistilled or deionised water** into each vial of the **Standards A-B**, cap the vial and **mix gently** to dissolve. Let stand for 10 min. Do not vortex or mix vigorously as this could cause denaturation of serum IgG and result in abnormally high values.	Storage: 2-8°C Stability: 5 d

Preparation of Working-Strength Standards

Directly prior to testing dilute **reconstituted** Standards 1:5 with **Sample Diluent**, e.g. 50 µL with 200 µL.	Storage: 2-8°C Stability: 48 h

TEST PROCEDURE

1.	Select sufficient microwells. Immediately prior to commencing the assay, wash plate 3x with 250 µL of prepared Wash Buffer. Remove excess solution by tapping the inverted plate on a paper towel, but do not allow plate to dry out.
2.	Pipette **100 µL** of each **Standard, Control and sample** into the respective wells of the microtiter plate. To achieve blanking a zero control of 100 µl Sample Diluent should be run as well.
3.	**Incubate 30 min at RT (18–24 °C).**
4.	**Wash 3 x** with **250 µL** of diluted **Wash Buffer**. Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
6.	**Incubate 15 min at RT (18–24 °C)**
7.	**Wash 3 x** with **250 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
8.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
9.	**Incubate 15 min at RT (18–24 °C).**
10.	**Stop the substrate reaction by adding 50 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
11.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **15 min** after pipetting of the TMB Stop Solution.

QUALITY CONTROL

The test results are valid only if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards and kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

It is recommended to participate at appropriate quality assessment trials.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage

conditions, pipettes, devices, incubation conditions and washing methods.

CALCULATION OF RESULTS

The blank corrected ODs of the standards (y-axis, linear) are plotted against their concentration (x-axis, linear) either on graph paper or using an automated method. The standard curve is calculated by a linear regression or a weighted linear regression function. Using computer programs, the curve is best described by a 2-point linear regression fit with linear axes. The assay can be declared valid if the following criteria are met: Slope: > 0.008; Y-intercept: < 0.600.
The concentration of the samples can be read directly from the standard curve.

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Typical Example

	Mean OD	C3d (µg/mL)
Blank	0.056	0
Standard 1	0.300	8
Standard 2	1.270	114
Sample 1	1.187	96.1
Sample 2	0.800	55.8

Slope: 0.0096
Y-Intercept: 0.171

LIMITATIONS OF THE PROCEDURE

C3d-containing CICs occur at low levels in other autoimmune and non-autoimmune conditions.
Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

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The following blood components do not have a significant effect on the test results up to the below stated concentrations:

EDTA	**50 mM**
Hemoglobin	**10 g/L**
Bilirubin	**0.25 g/L**

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