Complement C3a-desArg

ELISA

Enzyme Immunoassay - quantitative

Cat No.: PR 59011
Kit Size: 12 x 8 Determinations
Storage: 2-8°C
For in vitro use only

Intended Use
The PROGEN Complement C3a Enzyme Immunoassay measures the amount of C3a-desArg in human EDTA plasma, serum, and other biological and experimental samples.

Summary and Explanation
PROGEN's C3a Enzyme Immunoassay (ELISA) detects complement component C3a-desArg highly selectively using monoclonal antibodies. C3a is generated during activation of the complement system via the classical or the alternative pathway. The anaphylatoxin C3a itself is very short-lived and in the serum is cleaved immediately into the more stable C3a-desArg. Therefore, quantitation of C3a-desArg allows reliable measurement of the level of complement activation in the test sample.

Advantages of the Test Procedure
- Monoclonal antibodies are used for the determination and, thus, an optimum standardization and reproducibility is guaranteed.
- 8-well microtiter strips are used to allow optimal user flexibility.
- Micromethod, i.e. only 10 µl EDTA plasma is required per patient.
- Test results are available within approx. 2 - 3 hours.
- The test can be started immediately with the diluted sample (EDTA plasma), i.e. pretreatment of serum to remove native C3 is not required.

Description of the Method
- The microtiter strips included in the kit are coated with a monoclonal antibody specific for C3a-desArg.
- Plasma samples are diluted 1:100 (100 µl/well) and incubated for one hour at room temperature (RT; 18 - 25°C).
- After rinsing off the unbound native C3, peroxidase-conjugated mouse monoclonal antibody is used for the detection of C3a.
- Excess conjugate is removed through a washing step and the amount of C3a-desArg in the plasma sample is quantified using the peroxidase reaction.

Contents of the Kit
96-well microassay plate with 12 x 8-well microtiter strips, coated with monoclonal antibody to C3a
H 6 vials human C3a-desArg standard (1100 ng/ml), lyoph.*
N 2 vials negative control (human; C3a-desArg content < 150 ng/ml), lyoph.*
WB 1 bottle wash buffer concentrate (20x), 20 ml****
SB 2 bottles sample buffer concentrate (10x), 2 x 20 ml****
C 1 vial mab anti-C3a peroxidase conjugate (50x), 0.30 ml**/****
S 1 bottle substrate solution** (TMB, ready-to-use), 11 ml
SS 1 bottle stop solution (1N H2SO4), 13 ml***

* The sera have been tested for AIDS (HIV I+II), hepatitis B and syphilis and are negative. However, all human blood products should be considered to be potentially infectious.
** Light-sensitive; do not expose to bright light for prolonged period of time!
*** Avoid skin contact, contains diluted acid!
**** Toxic, contains 0.01% thimerosal!

INSTRUCTIONS FOR USE:
We recommend:
- Prepare buffer solutions only with distilled water.
- Do not let the wells dry out during the test procedure. The use of the plastic cover foil to cover the microtiter strips or a moist chamber is recommended for the incubation period.
- Use sterile disposable plastics for pipetting.
- Observe all pertinent precautions concerning the handling of potentially infectious material.

Warnings and Precautions
For in vitro Use Only

Safety Precautions
1. The standard and control contain human plasma which have been tested for hepatitis B surface antigen and HIV antibodies and was found to be negative. As no known test offers complete assurance that infectious agents are absent, the controls should be considered as potentially infectious and should be handled with the same precautions as any other potentially biohazardous material.
2. Do not pipette by mouth.
3. Do not smoke, eat, drink or apply cosmetics in areas where kits or serum samples are handled.
4. Any skin complaints, cuts, abrasions and other skin lesions should be suitably protected.
5. The kit Conjugate, Sample Buffer and Wash Buffer Concentrate contain thimerosal, which contains mercury and is toxic. When disposing of these reagents, observe authority regulations.
6. The Stop Solution contains sulfuric acid. Avoid contact with skin, eyes and mucous membranes. Accidental spillage should be mopped up with copious amounts of water. If contact with skin or eyes occurs, irrigate with water and seek medical attention immediately.

Specimen Collection:
Sample collection is critical. Care must be taken to avoid C3a generation in the sample.

All operations should be carried out at 2-8°C. Blood samples should be collected with disodium EDTA as anticoagulant and should be centrifuged immediately for 15 min at 2000x g at 2-8°C. Plasma should be isolated and assayed immediately or stored in aliquots at -70°C. The entire operations must be completed within 30 min.

Consequently, only EDTA plasma has to be used for the measurements which is either freshly collected or which has been stored prior at -70°C. Samples stored at 2-8°C for longer periods of time are obsolete. A correct sample collection is mandatory for the determination of C3a. If frozen, thaw samples quickly at 37°C and immediately transfer them to ice (do not leave at 37°C!)

The surface of various plastic materials can also induce complement activation, we, therefore, suggest to use glass tubes for all samples (not necessary for the standard and negative control).

Preparation of Reagents
- Allow kit components to reach RT.
- SB Sample Buffer
  To prepare ready-to-use sample buffer (SB), dilute the buffer 1:10 with distilled water. Example: empty the contents of one bottle SB into a glass cylinder, add distilled water to make up 200 ml and mix well by stirring. The buffer is now ready-to-use and for a minimum of 6 weeks stable at 2-8°C.
- Wash Buffer
  For preparing the ready-to-use wash buffer (WB), dilute 1:20 with distilled water. Example: empty the contents of the bottle WB into a glass cylinder, add distilled water to make up 400 ml and mix well by stirring. The buffer is now ready-to-use and for a minimum of 6 weeks stable at 2-8°C.

ATTENTION: We recommend to mark the diluted buffers unambiguously. Sample buffer and wash buffer must not be exchanged!
- Standards
  a) Dissolve the positive standard H in 1 ml ready-to-use sample buffer SB, mix carefully (by slowlypipetting 3x up and down the vial; do not vortex!) and incubate for maximum 5 min at RT. Prepare the standard dilutions H/2, H/4 and H/8 with H. Example: take 500 µl sample buffer and mix with 500 µl H (= H/2); take 500 µl sample buffer and mix with 500 µl H/2 (= H/4); etc.
  b) Dissolve negative control plasma N in 1 ml ready-to-use sample buffer SB, mix carefully and incubate for maximum 5 min. The negative control is now ready-to-use. For further test runs, the negative control can be aliquoted and stored at -20°C. Do not store reconstituted or diluted standards. Use immediately after preparation. Discard after use.
- Anti-C3a Peroxidase Conjugate
  The conjugate concentrate has to be diluted 1:50 in ready-to-use sample buffer (SB). As the diluted solution is not stable, it is recommended to always only prepare the required conjugate dilution.
- Substrate Solution
  The Substrate Solution is provided in ready-to-use form. If stored properly at 2-8°C and not exposed to bright light for prolonged periods of time, the solution is stable until the expiration date printed on the label.

Test Procedure
If required, let plasma sample thaw and keep on ice. Dilute plasma samples 1:100 (10 µl for 1 ml) in sample buffer.
- Sample Incubation: According to the pipetting scheme, pipette 100 µl each of ready-to-use sample buffer (blank), standard, control and diluted (patient) samples (1:100) into the individual wells and incubate 1 h at RT. We recommend double determinations.
* Washing: Empty microtiter strips and pipette 200 µl each of ready-to-use wash buffer into the wells. After max. 1 min, empty wells again and repeat this washing step two more times. Remove excess liquid by tapping the strips on absorbent paper.

* Conjugate Incubation: Pipette 100 µl of the diluted conjugate solution into each well and incubate 1 h at RT.

* Washing: Empty microtiter strips and carry out washing steps as described above.

* Substrate Incubation: Pipette 100 µl substrate solution into the wells and incubate 10 to 15 min.

* Stopping Reaction: Pipette 100 µl SS into the wells and maintain same sequence as before when adding the substrate solution.

Measuring the Absorbance (A)
- Measure the Absorbance with a microtiter plate reader at 450 nm wavelength and a reference wavelength of 600 - 650 nm (single-beam equipment may also be used). Well A1 (substrate control) serves as BLANK.
- If in the individual wells an A of > 2.0 is measured, the sample has to be diluted further and measured again.

Calculation of Results
Plot the net absorbance values for each C3a standard concentration on the y-axis for each corresponding C3a concentration on the x-axis (see: Example of Standard Curve).

The C3a concentration in each unknown sample can be determined directly by reading the C3a concentration corresponding to the absorbance value (i.e. the dilution factor has already been included in the concentration values of the x-axis). If a dilution greater than x100 has been chosen, only the additional dilution factor has to be included for calculation! We recommend to apply a 4-parameter calculation mode to determine the best fit line. Most software is programmed for this purpose and may be used to determine the concentration (ng/ml) in each sample as well.

Expected Values/Interpretation
From a standard collective of normals, (20 blood donors) a C3a-desArg content of 150 ng/ml plasma was determined. Titers are elevated if plasma samples are > 200 ng/ml.

References