5-ELISA ANTI-ANNEXIN A1, IgG Autoantibody

Ref# 5D-55904

ELISA for measurement of ANTI-ANNEXIN A1, IgG

96 tests micro-ELISA plate

5-Diagnostics

Research Use Only. Not for use in diagnostic procedures

Intended use:

The 5-ELISA Anti-ANNEXIN A1, IgG kit is a two-site enzyme immunoassay for measuring Anti-Annexin A1, IgG autoantibodies in plasma or in any biological fluid where they must be tested.

Summary and explanation:

This sandwich ELISA is designed with recombinant Annexin A1 antigen coated onto the plate for capturing Anti-ANNEXIN A1 IgG autoantibodies in the tested sample. Following a washing step, captured IgG autoantibodies are tagged with a peroxidase-labelled polyclonal antibody, which binds onto them in a dose-dependent manner. After washing away, the excess of immunoconjugate, the substrate, 3,3',5,5' Tetramethylbenzidine (TMB) with hydrogen peroxide (H_2O_2) is introduced and a blue color develops, which turns yellow when the reaction is stopped with sulfuric acid. This color is measured at 450 nm, and is directly proportional to the amount of anti-ANNEXIN A1 autoantibodies present in the tested sample.

Annexin A1, or Lipocortin I, is a 37 kDa protein from the annexin family with strong anti-inflammatory and pro-resolving activities (Perretti and D'Aquisto, *Nat Rev Immunol* 2009). Annexin A1 is highly expressed in neutrophils, monocytes and macrophages and modestly in mast cells, endothelial cells, epithelial cells and fibroblasts. Autoantibodies to Annexin A1 have been reported in some pathological conditions.

Tested samples:

- Citrate or Na_2 EDTA anticoagulated human plasma, or human serum.
- Any biological fluid, where human auto-antibodies to ANNEXIN A1, of the IgG isotype, must be assayed.

Reagents:

1. **COAT**: Micro ELISA plate, containing 12x8 well strips, coated with Annexin A1 recombinant protein, stabilized; the plate is packed in an aluminum pouch sealed in presence of a desiccant.
2. **SD**: 2 vials of 30 mL Sample Diluent containing 10% Goat serum, ready to use: this solution contains a high NaCl concentration.
3. **WS**: 50 mL vial of **20-fold** concentrated Wash Solution.
4. **C+**: 2 vials of lyophilized Positive Control Plasma, ***already diluted 1:100*** when restored with 1 mL of SD.
5. **C-**: 2 vials of lyophilized Negative Control Plasma, ***already diluted 1:100*** when restored with 1 mL of SD.
6. **IC**: 500 µL vial of Goat Anti-(human)-IgG-Peroxidase conjugate: **50-fold** concentrated.
7. **ICD**: 25 mL of Conjugate Diluent, ready to use.
8. **TMB**: 26 mL of Peroxidase Substrate, 3,3',5,5'-Tetramethylbenzidine containing hydrogen peroxide. Ready to use.
9. **SA**: 7.5 mL vial of 0.50 M Sulfuric Acid, ready to use.

Warning and cautions:

- Some reagents provided in this kit contain materials of human (control plasma) and animal (BSA, goat serum) origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative.

The bovine blood used to prepare BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.

However, no test method can offer complete assurance that infectious agents are absent. Therefore, laboratory operators must exercise extreme when using these reagents, in full compliance with safety cautions for the manipulation of biological materials, and treat them as if they were infectious.

- Waste should be disposed in accordance with applicable local regulations.
- Use only reagents from the same kits' batch.
- Any incident that has occurred in relation with the device use shall be reported to the manufacturer.
- If the TMB substrate becomes yellow, this indicates the presence of a contaminant, and it must be rejected.

Reagent preparation:

Bring the kit at room temperature, at least 30 minutes before use, to avoid use of reagents at a too low temperature, which could reduce the assay kinetics.

Store unused reagents at 2-8°C.

Lyophilized vials are closed under vacuum. Carefully remove the stopper to avoid any loss of powder when opening these vials.

When appropriately used and stored according to the recommended protocol and cautions, the kit content can be used over a 1-month period following first use, and strip by strip, if required.

1. COAT (Micro ELISA plate): Open the aluminum pouch and take off the required number of 8-well strips for the test series. When out of the pouch, the strips must be used within 30 minutes.
   - Unused strips can be stored at 2-8°C for 4 weeks in their original aluminum pouch, in presence of the desiccant, hermetically closed with the minigrip system and protected from any moisture.
2. **SD** (Sample Diluent): Ready to use.
   This reagent contains 0.05% Proclin-300, and goat serum to minimalize the interference of sticky components or heterophilic antibodies. Provided that any contamination or evaporation are avoided, and kept in its original vial duly closed, stability after opening is of:
   - 4 weeks at 2-8°C.
3. **WS** (Wash Solution): If necessary, incubate the vial in a water bath at 37°C, until complete dissolution of solids. Shake the vial and dilute **20-fold** the required volume with distilled water (the 50 mL contained in the vial allow preparing 1 liter of Wash Solution). Provided that any contamination or evaporation are avoided, and kept in its original vial, stability after opening is of:
   - 4 weeks at 2-8°C.
4,5. **C+, C-** (Positive and Negative Control Plasmas): Reconstitute each vial with 1 mL of **SD** sample diluent to get **100-fold** diluted control plasmas (C+ and C-), ready to use.
   - 8 hours at room temperature (18-25°C).
   - 24 hours at 2-8°C
   - 2 months at ≤ -20°C.
6. **IC** (Goat Anti Human IgG-HRP conjugated): Stability after opening and provided that any contamination or evaporation is avoided, kept in its original vial:
   - 4 weeks at 2-8°C.

   Just before use, dilute **50-fold** the requested volume of the concentrated **IC** (50x) with the **ICD** "Conjugate Diluent" and shake to homogenize.
   Stability of diluted Conjugate:
   - 6 hours at room temperature (18-25°C).
7. **ICD** (Immuno-Conjugate Diluent): Ready to use. This reagent contains 0.05% Proclin-300. Stability after opening, and provided that any contamination or evaporation is avoided, kept in its original vial:
   - 4 weeks at 2-8°C.
8. **TMB**: Ready to use. Kept in its original vial and provided that any contamination or evaporation are avoided, stability of reconstituted substrate is of:
   - 4 weeks at 2-8°C.
9. **SA** (Stop Solution): Stop solution containing 0.45M sulfuric acid, ready to use.

Reagents and materials not provided:

- 8-channel or repeating micro-ELISA pipette for volumes of 50-300 µL.
- Pipettes at variable volumes from 0 to 20, 20 to 200 and 200 to 1000 µL.
- Micro ELISA plate reader with a wavelength set up at 450 nm.

Specimen collection and preparation:

Blood (9 volumes) should be carefully collected onto 0.109M (3.2%) trisodium citrate anticoagulant (1 volume) by clean venipuncture. Serum can be prepared according to local guidelines.

Samples should be collected, prepared, and stored in accordance with applicable local guidelines.

Assay method:

The standard assay protocol recommends a **1:100** plasma dilution with the **SD** sample diluent. If very high concentrations of anti-ANNEXIN A1 autoantibodies are suspected, a complementary dilution factor must be used. Results must be then corrected with the additional dilution factor.

The assay is semi-quantitative, and results are expressed in A450, and compared to the mean value and 2 or 5 Standard Deviations (SDs) obtained on at least 30 normal plasmas.

Controls are ready to use (already diluted **1:100**).

4. Procedure:

Remove the required number of strips from the aluminium pouch and place them into the frame provided. Introduce the reagents in the microplate wells and perform the assay as indicated on the here below table:

Samples or Reagent	Volume	Protocol
Controls (C+, C-) or diluted tested specimen	200 µL/well	Introduce rapidly into microwells **(a)**
*Incubate for 60 minutes at RT (18-25 °C) **(b) (c)***		
WS	300 µL/well	5 successive washing steps
IC (50-fold diluted in ICD)	200 µL/well	Introduce immediately after washing **(d)**
*Incubate for 60 minutes at RT (18-25°C) **(b) (c)***		
WS (20-fold diluted with purified water)	300 µL/well	5 successive washing steps
TMB-H_2O_2	200 µL/well	Introduce immediately **(d)**
*Incubate for exactly 5 minutes at RT (18-25 °C) **(b) (c)***		
SA (e)	50 µL/well	Stop after exactly 5 minutes
Homogenize by shaking smoothly and wait for 10 minutes Read the absorbance at 450 nm within 20 minutes against the blank or subtract the blank value obtained with SD alone (f, g)		

Notes:

a) Distribute controls and specimen as rapidly as possible (within 10 minutes), to obtain homogeneous immunological kinetics. A too long delay between the distribution of the first and the last wells may influence immunological kinetics and generate inaccurate results (last wells distributed underestimated).
(b) Avoid letting the plate at the bright sunlight during incubations, and more particularly during colour development.
(c) Perform the incubations preferably at 20±1°C.
(d) Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within less than 3 minutes, to prevent the plate from drying, which could damage the immobilized components and reduce reagents' reactivity. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted to wash the plates gently, and to avoid a too drastic emptying, which could damage coating and lower plate reactivity.
(e) For addition of the substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.
(f) For bichromatic readings, a reference wavelength from 620 nm to 690 nm can be used.
(g) The A450 blank value must be subtracted from the A450 values measured for tested samples. Alternatively, the reader zero must be adjusted on the blank (SD alone).

Quality control:

Using quality controls allows validating the method compliance, as well as the homogeneity of assays for a same lot of reagents
Quality control must be included in each series, as per good laboratory practice, to validate test results.
Each laboratory should establish acceptance ranges and verify expected performances in its analytical system. In case of assay automatization, the user agrees to conduct a complete study to validate the analytical performance according to the standards in force.

Results:

Results are expressed with the measured A450, and compared to the normal range (Mean + 2 SDs).
Absorbances can slightly vary from lot to lot, according to the anti-IgG-peroxidase lot used. As an example, the A450 threshold values for the negative, doubtful and positive ranges, are indicated on the lot-specific flyer included in the kit.
For example:

Negative range: A450 ≤ 0.30
Positive range: A450 ≥ 0.50
Grey zone: A450 > 030 and < 0.50

Alternatively, an ELISA software (i.e., Dynex, Biolise, etc.) can be used for expressing the results.

The results obtained should be for research use only and must not be used for patient diagnosis or treatment.

Limitations:

- To ensure optimum test performance and to meet the specifications, the technical instructions should be followed carefully.
- Any reagent presenting no clear appearance or showing signs of contamination must be rejected.
- Any suspicious samples must be rejected.
- If the washing step is not correctly performed, this can produce a high absorbance value, especially for blanks. To avoid non-specific color development, check that the washing step is performed efficiently.
- It is the responsibility of the user to validate any modification to these instructions of use.
- Erroneous results can occur from: bacterial contamination of reagents; inadequate incubation steps; inadequate washing of tested wells; exposure of substrate to bright light/sunlight; omission of a test reagent; exposure to temperatures higher or lower than prescribed; requirements or omission of steps.
- Goat serum is added to the sample diluent (SD) to avoid or minimalize non-specific binding, and to offer the highest specificity grade.

Performances:

As an example, for lot 240129-10, when tested at 20 +/- 1°C, the following results are obtained:

Inter-assay Mean and CV:
C+: A450=2.80; Inter-assay CV=4.5%; Intra-assay CV= 3.21%
C-: A450=0.16; Inter-assay CV=6.3% ; Intra-assay CV= 4.34%

Normal concentration in plasma:

Anti-ANNEXIN A1 IgG autoantibodies are normally absent from plasma or serum in healthy population.

Additional information:

Annexin A1 is a member of the annexin protein family. Like most members it binds negatively charged phospholipids in Ca^{2+}-dependent manner. In addition, it binds the FPR2/ALX receptor and elicits anti-inflammatory responses upon FPR2/ALX ligation. Annexin A1 is regarded as an important mediator of resolution of acute inflammation. Blood plasma Annexin A1 originates mostly from activated and dying cells of the innate and adaptive immune system, including neutrophils, monocytes, macrophages, and dendritic cells. Plasma Annexin A1 reflects ongoing inflammation and an activated immune system, especially in cancer. Plasma Annexin A1 can be proteolytically cleaved into an N-terminal domain of around 3 kDa and a C-terminal domain of circa 34 kDa. Plasma Annexin A1 is a useful biomarker for immune-inflammatory states associated with infectious diseases, malignancy and autoimmunity.

Nota:

When IgM or IgA isotypes' anti-ANNEXIN A1 autoantibodies must be investigated, 50-fold anti-IgM-HRP or anti-IgA-HRP conjugates, specific for human IgMs or human IgAs, are available upon request.

References:

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