

Test Instructions IMTEC-Annexin V-Antibodies Screen

Enzyme Immunoassay for the Quantitative Determination of IgG, IgM and IgA Antibodies against Annexin V

REF : TC 59550

Please read the instructions carefully before testing.

Procedural precautions:

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different lots.
- ▶ Store reagents at 2-8°C.

1. Clinical Use

In the widest sense, anti-annexin antibodies are included in the family of antiphospholipid antibodies typically associated with primary and secondary antiphospholipid syndrome (APS), an autoimmune disease characterized by venous and arterial thrombosis, recurrent miscarriage and other clinical symptoms.

Annexin V, a member of the annexin family, is an effective and potent anticoagulant protein. Its activity is based on high-affinity binding to anionic phospholipids, leading to the inhibition of the phospholipid-dependent coagulation cascade. The protein also seems to have a thrombomodulatory function in the placental circulation since it is necessary for the preservation of the placental integrity.

Inhibition of annexin V function can be achieved either through autoantibodies against the protein alone or through autoantibodies directed against a complex of (anionic) phospholipids and annexin V (cofactor).

Antiphospholipid antibodies are thought to neutralize the anticoagulatory protective function of annexin V in the placenta, resulting in placental thrombosis and detachment. Autoantibodies against annexin V have been primarily described in women with a history of recurrent fetal loss, intrauterine death and premature delivery. Besides occurring in SLE patients with recurrent fetal loss, anti-annexin V antibodies have also been detected in women with a history of recurrent miscarriage with no signs of thromboembolic complications.

It is therefore especially important to test for anti-annexin V antibodies in women with an increased risk of prenatal complications in the context of diagnostics for APS as well as in cases of recurrent fetal loss of unclear etiology.

2. Principle of the Test

The test is based on the immobilisation of annexin V to a solid phase (polystyrene) and subsequent binding of the autoantibodies. For the detection of the bound antibodies an enzyme-labeled second antibody is used which is directed against human IgG, IgM and IgA conjugated to the enzyme peroxidase. After addition of a substrate solution, a color develops, its intensity is proportional to the concentration and/or the avidity of the antibodies.

3. Materials Provided

-	MTP : microtiter strips (1x8), ready to use, breakable, coated with annexin V	12 strips + frame
-	CAL : standards, ready to use	750 µl per vial 1 vial each
	1 6.25 U/mL	
	2 12.5 U/mL	
	3 25 U/mL	
	4 50 U/mL	
	5 100 U/mL	
	(all standards contain sodium azide and are inked according to concentration)	
-	CONTROL - : negative control serum ready to use, contains sodium azide	1 vial 1 ml
-	CONTROL + : positive control serum, ready to use, contains sodium azide	1 vial 1 ml
-	BUF WASH 10x : washing buffer concentrate (10x)	1 bottle 50 ml
-	DIL SPE : sample buffer, ready to use, contains sodium azide	1 bottle 100 ml
-	CONJ a(hum Ig(GAM)):HRP : HRP-Conjugate, anti-human IgGAM, ready to use	1 bottle 12 ml
-	SUBS TMB : TMB solution, HRP substrate, ready to use	1 bottle 12 ml
-	SOLN STOP : stopping solution, ready to use, contains sulfuric acid, caution corrosive!	1 bottle 12 ml

4. Preparation of Reagents

Attention!

Allow the testkit and all its components to reach room temperature completely before executing it!

Please do not use any polystyrene vessels for handling of HRP conjugates.

If the test is running automatically, it is recommended to use fresh conjugate each time. Please remove traces of old conjugate completely.

4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate [BUF] [WASH] [10x] with 9 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2-8°C.

4.2. Standards, Control Sera, Sample Buffer, HRP Conjugate, Stopping Solution and TMB Solution

Standards, control sera, sample buffer, HRP-Conjugate, stopping solution and TMB solution are ready to use. Used bottles should be closed carefully and stored at 2-8°C. **Store TMB solution also protected from light.**

4.3. Preparation of Samples

Use serum or plasma samples freshly collected or freeze samples at -20 °C. Do not use samples, that are repeatedly thawed and frozen. Do not use serum samples inactivated by heat treatment at 56 °C. Allow the samples to reach room temperature (30 min). Dilute samples 1 : 100 with sample buffer [DIL] [SPE] (10 µl sample to 1 ml buffer).

4.4. Microtiter Strips

The strips are ready to use. Unused strips should be stored in the lockable original bag at 2 – 8 °C.

5. Test Procedure

- **Pipette 100 µL serum dilution** or standards [CAL] (inked according to rising concentration) or control sera [CONTROL] [+] and [CONTROL] [-], resp., into each well, for blank use sample buffer [DIL] [SPE] instead of serum dilution, seal wells with adhesive foil.
- **Incubate for 1 hour** at room temperature (RT).
- **Rinse the wells 3 x** using at least 200 µl washing buffer per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µL of HRP-conjugate** [CONJ] [a(hum Ig(GAM)):HRP] into each well, seal wells with adhesive foil.
- **Incubate for 30 minutes** at RT.
- **Rinse the wells 3 x** using at least 200 µl washing buffer per well.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.

- **Pipette 100 µL TMB solution** [SUBS] [TMB] into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 µL stopping solution** [SOLN] [STOP] per well.
- **Measure at 450 nm** within the next 30 min after stopping.

6. Interpretation of Results

Calibrate measured absorbances against concentrations/units of standards [CAL] (6.25 U/mL, 12.5 U/mL, 25 U/mL, 50 U/mL, 100 U/mL) in semilog. Determine the units of the examined sera from the standard curve directly.

Results above 25 U/mL (cut-off value) for anti-annexin V-antibodies are considered positive.

To prove the functionality of the test, the determined value for the positive control serum [CONTROL] [+] is to be expected within the range labeled on the vial. The result of the negative control [CONTROL] [-] has to be lower than the cut-off value of the test.

Precautions

For in vitro diagnostic use only.

[IVD]

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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