Human ACPA ELISA Kit

Enzyme Immunoassay for the quantification of Human ACPA (anti–citrullinated protein antibody) in human serum or plasma.

Catalog number: ARG80351

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Anti-citrullinated protein antibodies (ACPAs) are autoantibodies (antibodies to an individual's own proteins) that are directed against peptides and proteins that are citrullinated. They are present in the majority of patients with rheumatoid arthritis. Clinically, cyclic citrullinated peptides (CCP) are frequently used to detect these antibodies in patient serum or plasma (then referred to as anti–citrullinated peptide antibodies).

During inflammation, arginine amino acid residues can be enzymatically converted into citrulline residues in proteins such as vimentin, by a process called citrullination. If their shapes are significantly altered, the proteins may be seen as antigens by the immune system, thereby generating an immune response. ACPAs have proved to be powerful biomarkers that allow the diagnosis of rheumatoid arthritis (RA) to be made at a very early stage.

In July 2010, the 2010 ACR/EULAR Rheumatoid Arthritis Classification Criteria were introduced. These new classification criteria include ACPA testing, and overruled the "old" ACR criteria of 1987 and are adapted for early RA diagnosis.

[Provide by Wikipedia: Anti–citrullinated protein antibody]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. Human ACPA ELISA Kit is a test system for the quantitative measurement of IgG class autoantibodies against mutated citrullinated vimentin (MCV) in human serum or plasma. A highly purified cyclic citrullinated vimentin peptides has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any citrullinated protein antibodies (Ab) present is bound by the

immobilized antigen. After washing away any unbound substances, a HRP-conjugated human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of citrullinated protein Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C.
Standard 0-5 (0, 20, 40, 100, 300, 1000 U/ml)	6 X 1.5 ml (Ready-to-use)	4°C.
Control 1 (Positive Control; acc. range: 220 - 380 U/ml)	1 X 1.5 ml (Ready-to-use)	4°C
Control 2 (Negative Control; acc. range: < 20 U/ml)	1 X 1.5 ml (Ready-to-use)	4°C
5X Sample buffer	20 ml	4°C
HRP-Antibody conjugate	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: reference filter at 620 nm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C in the dark at all times. Do not expose reagents to heat, sun, or strong light during storage and usage.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- All materials must be at room temperature (20-28°C) prior to use.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- All incubation steps must be accurately timed.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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 for up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, Citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C for up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
b) Samples containing sodium azide should not be used in the assay.
c) Testing of heat-inactivated sera is not recommended.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute **50X** Wash buffer into **distilled water** to yield 1X Wash buffer. (E.g. 20 ml of 50X Wash buffer + 980 ml of distilled water) The diluted Wash buffer is stable for 30 days at 2°C to 8°C.
- **1X Sample buffer:** Dilute **5X** Sample buffer with distilled water before use. (E.g. 20 ml of 5X Sample buffer + 80 ml of distilled water) The diluted Sample buffer is stable for 30 days at 2°C to 8°C.

- **Patient sample**: Dilute patient sample **1:100** with **1X sample buffer** before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)

  Note: the controls and calibrators are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-28 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards, controls and prediluted samples** into wells.
3. Incubate for **30 minutes at RT**.
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of **HRP-Antibody conjugate** solution into each well. Incubate for **15 minutes at RT**.
6. **Wash** as according to step 4.
7. Add **100 µl** of **TMB Reagent** to each well. Incubate for **15 minutes at room temperature in dark**.
8. Add **100 µl** of **Stop Solution** to each well. **Incubate for 5 minutes at RT**. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at **450 nm** immediately (optional: Read the OD at 600-690nm as reference). It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If these quality control criteria are not met the assay run is invalid and should be repeated.

7. EXPECTED VALUES

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 20 U/ml

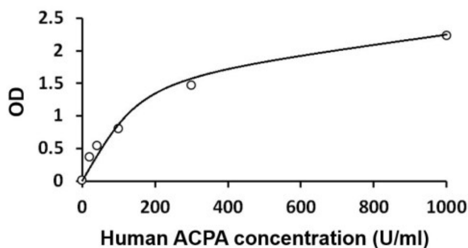
8. INTERPRETATION OF RESULTS

Negative: < 20 U/ml

Positive: ≥ 20 U/ml

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



The OD $_{450/620nm}$ value of standard 0 (0 U/ml) should < 0.15

The OD $_{450/620nm}$ value of standard 5 (1000 U/ml) should > 1.3

QUALITY ASSURANCE

Sensitivity

The standard range of ACPA ranged from 20-1000 U/ml. The mean Limit of detection was 1 U/ml.

Interfering substance

No interference has been found with hemolytic or lipemic sera, or bilirubin-containing sera.

Linearity

95%- 120%

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 10.4% and inter-assay precision was 7.5%.