Human Anti-Carbamylated Protein Antibody, (Anti-CarP) ELISA Kit

Catalog number: BG-HUM09021 (96 wells)

The kit is designed to detect the presence of anti-CarP autoantibody in serum, plasma and other suitable sample solution

FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC PURPOSES
**Intended Use**

The Anti-CarP test kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of IgG antibodies to carbamylated proteins in human serum or plasma. The assay is used to detect antibodies in a single specimen. The results of the assay are to be used as an aid to the diagnosis or research of Rheumatoid Arthritis (RA), in conjunction with other laboratory and clinical findings. The analysis should be performed by trained laboratory professionals. For in vitro detection use.

**Background Information**

Rheumatoid Arthritis (RA) is one of the most common systemic autoimmune diseases. The aetiology of the disease, which affects up to 1-2% of the world population, is unknown. The diagnosis of RA depends primarily on clinical manifestation of the disease. The only serological test routinely used is the determination of the presence of rheumatoid factors (RF) in the serum. RF are antibodies directed to the constant region of immunoglobulins of the IgG class. However, these antibodies are also present in relatively high percentages in other autoimmune diseases, infections and in up to 15% of healthy individuals. Antibodies of a more specific nature have also been found in sera of RA patients Anti-perinuclear factor (APF) antibodies are reported to be present in around 50% of RA patients with a specificity of over 70%. A number of cyclic synthetic peptides not related to filaggrin or other known proteins have been described which are specifically recognized by autoantibodies in sera from RA patients. These peptides were subsequently used in an ELISA for the detection of RA-specific autoantibodies. Clinical evaluation studies showed that the ELISA was positive in a significant number of well-defined RA patient sera with a high specificity against disease controls. A diagnostic and prognostic value for the measurement of the anti-carbamylated protein (anti-CarP) antibodies was found in relation to joint involvement and radiological damage in early RA. This Anti-Carbamylated Protein Antibody assay is based on highly purified synthetic peptides containing carbamylated residues and is an additional tool in the diagnosis of RA. This anti-Carb kit contains improved synthetic peptides selected on the basis of performance in the detection of RA autoantibodies.

**Principle of the Assay**

The anti-CarP antibody kit is based on an ELISA method. The test utilizes microtitre wells coated with carbamylated synthetic peptides (antigen). Diluted patient serum or plasma is applied to the wells and incubated. If specific antibodies are present, they will bind to the antigen in the wells. Unbound material is washed away and any bound antibody is detected by adding biotin labelled antibodies to human IgG, followed by a second washing step, followed by incubation with streptavidin-HRP, washing and incubation with a chromogenic substrate. The presence of reacting antibodies will result in the development of color, which is proportional to the quantity of bound antibody, and this is determined photometrically.
**Kit Components**

1. Purified Anti-Carb antibody reference*: 1 vials (ready to dilute)
2. 96-well plate pre-coated with Carb: 1
3. Sample Diluent Buffer: 12 ml × 2
4. Detection antibody-biotin: 1 vial, 60 μl
5. Streptavidin-HRP: 1 vial, 150 μl
6. Positive control: 1 ml, 1x.
7. Negative control: 1 ml, 1x.
8. Antibody and HRP diluent: 12 ml × 2
9. TMB substrate: 8 ml
10. Stop Solution: 6 ml
11. 20 × Wash Buffer: 25 ml
12. Plate sealers: 2
13. Package insert: 1

*1x anti-CarP antibody reference containing 100 ng/ml of purified rabbit anti-CarP antibody. Please do serial dilution as signal intensity reference.

**Materials required but not supplied**

- 1x PBS.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

**Sample Preparation and storage**

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
• Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 2000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

• Plasma: Collect plasma. Centrifuge for 15 min at 2000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at 20°C.

Sample Dilution:

Please first try 1:200 sample dilution in sample diluent. Then change the dilution factor based on the results.

Reagent Preparation

Detection Antibody

Dilute 30 μl of 200x anti-human antibody-biotin into 6 ml of Antibody Dilution Buffer.

Streptavidin-HRP

Dilute by mixing 150 μl of the 40x solution with 5.85 ml of the Antibody Dilution Buffer.

Wash Buffer

• If crystals have formed in the 20X wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

• Dilute 25 ml Wash Buffer Concentrate (20X) to a total volume of 500 ml with distilled water.

Assay Procedure

Bring all reagents to room temperature before use. Human Anti-Carb standard curve should be prepared for each experiment. The user will decide sample dilution factor by rough estimation of Human Anti-Carb concentration in samples.

1. Add 50 μl of diluted sample or standards or control per well. Add 0.1 ml of the sample diluent into the control well (Zero well). Cover with an adhesive strip and incubate at room temperature for 1 hour. Note: We recommend that each standard solution and each sample is measured in duplicate.

2. Aspirate each well and wash with Wash Buffer, repeating the process four times for a total of 5 washes. Wash by filling each well with Wash Buffer (300 μl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.

3. Add 50 μl of the Detection Antibody working solution to each well. Cover with a new adhesive strip and incubate at room temperature for 60 min.

4. Repeat the aspiration/wash as in step 2.
5. Add 50 µl of streptavidin-HRP working solution to each well. Cover with a new adhesive strip and incubate at room temperature for 30 min.

6. Repeat the aspiration/wash as in step 2.

7. Add 50 µl of TMB substrate solution to each well. Cover and incubate at room temperature for 15 to 20 min or until a gradient develops and you see visible color in the 2nd lowest concentration well. Protect from light. Do not over-develop.

8. Add 50 µl Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

**Calculation and Interpretation of Results**

Consider each assay separately when calculating and interpreting results.

**Qualitative protocol:**

Calculate the absorbance value (optical density) ratio for the Positive and Negative Controls, and for each sample:

\[ \text{Absorbance Ratio} = \frac{\text{Sample or Control Absorbance Value}}{\text{mean Reference Control Absorbance Value}} \]

Users should calculate a cut-off between positive and negative samples that is specific to their patient populations. Results from the patient populations used in this kit suggest the following cut-off:

<table>
<thead>
<tr>
<th>Absorbance Ratio</th>
<th>Results Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.95</td>
<td>Negative</td>
</tr>
<tr>
<td>&gt;=0.95 to &lt;= 1.0</td>
<td>Bordline - recommend repeat testing</td>
</tr>
<tr>
<td>&gt; 1.0</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**Precision**

Intra-Assay CV: 1.1 to 7.5%, average 3.7%

Inter-Assay CV: 8.2 to 11.2 %, average 9.6%

**Dilution Recovery of serum**

81% to 113 %,