Anti-Ribosomal P (IgG) ELISA

For the quantitative and qualitative detection of antibodies against ribosomal P-proteins (rib-P) in serum.

Catalog Number: 35-RIBHU-E01
Size: 96 wells

For Research Use Only. Not For Use In Diagnostic Procedures.

ALPCO Diagnostics

26G Keewaydin Drive • Salem, NH 03079
Phone: (800) 592-5726 • Fax: (603) 898-6854
www.alpco.com • Email: web@alpco.com
1. Intended Use

 Rib-P is a solid phase enzyme immunoassay employing native human ribosomal P-proteins P0, P1 and P2 isolated from eukaryotic cell line for the quantitative and qualitative detection of antibodies against ribosomal P-proteins (rib-P) in human serum. The specificity of anti-rib-P antibodies is restricted to a common antigenic determinant located on the highly conserved carboxyl-terminal portion of the three P proteins. The assay is a tool in the diagnosis of systemic lupus erythematosus (SLE).

2. Clinical Application and Principle of the Assay

The ribosomal phosphoproteins P0 (~38 kDa), P1 (~19 kDa) and P2 (~17 kDa) are located within the 60S subunit of human ribosomes. In contrast to the majority of basic ribosomal proteins, P1 and P2 are acidic. The ribosomal proteins are associated to a pentamer with two P1/P2 heterodimers anchored to P0 by the amino terminal portion of P2. This pentamer is located in a highly accessible region on the stalk of the ribosome. Biochemical studies suggest that P1/P2 play a fundamental role in all three phases of ribosomal polypeptid synthesis (initiation, translocation, termination).

Autoantibodies to ribosomal proteins are highly specific for SLE since they are not found in other autoimmune diseases or in infections. The frequency of anti-rib-P antibodies is 10-20% in randomly selected SLE patients. Anti-rib-P antibodies are detected more frequently in lupus patients with severe psychiatric manifestations. In addition, other organ involvement including renal and hepatic disease might be correlated with the presence of anti-rib-P.

**Principle of the test**

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient’s antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.
3. Kit Contents

To be reconstituted:
5x Sample Buffer  1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
                 Containing: Tris, NaCl, BSA, sodium azide < 0.1% (preservative)
50x Wash Buffer  1 vial, 20 ml - 50x concentrated (capped white: green solution)
                 Containing: Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)

Ready to use:
Negative Control 1 vial, 1.5 ml (capped green: colorless solution)
                 Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
                 Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
Cut-off Calibrator 1 vial, 1.5 ml (capped blue: yellow solution)
                 Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
Calibrators       6 vials, 1.5 ml each  0, 3, 10, 30, 100, 300 U/ml
                 (color increasing with concentration: yellow solutions)
                 Containing: Human serum (diluted), sodium azide < 0.1% (preservative)
Conjugate        1 vial,15 ml IgG (capped blue: blue solution)
                 Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase
TMB Substrate    1 vial, 15 ml (capped black)
                 Containing: Stabilized TMB/H2O2
Stop Solution    1 vial, 15 ml (capped white: colorless solution)
                 Containing: 1M Hydrochloric Acid
Microtiterplate  12x8 well strips with breakaway microwells
                 Coating see paragraph 1

Material required but not provided:
Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass
ware(cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500,
1000 µl) or adjustable multipipette (100-1000ml). Microplate washing device (300 µl repeating or multi-
channel pipette or automated system), adsorbent paper.
Our tests are designed to be used with purified water according to the definition of the United States
Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared,
reconstituted solutions are stable for 1 month at 4°C/39°F, at least. Reagents and the microplate
shall be used within the expiry date indicated on each component, only. Avoid intense exposure
of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal
tightly.
5. Precautions of Use

5.1 Health hazard data

**This product is for in vitro diagnostic use only.** Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

**Recommendations and precautions**

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

*WARNING!* Calibrators, Controls and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or adsorbed by skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit.

Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation: We recommend test performance at 30°C/86°F for automated systems.**

Never expose components to higher temperature than 37°C/ 98.6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35-46°F up to three days, or frozen at -20°C/-4°F for longer periods.
7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:
- Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
- Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples:
- Dilute serum samples 1:101 with sample buffer (1x)
  e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well!

Washing:
- Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells
  e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:
- Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:
- Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:
- Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Work flow

For pipetting scheme see Annex A, for the test procedure see Annex B
We recommend pipetting samples and calibrators in duplicate.
Cut-off calibrator should be used for qualitative testing only.

- Pipette 100 µl of each patient's diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off calibrator and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F.
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.
8. Quantitative and Qualitative Interpretation

For quantitative interpretation establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

![Normal Range](<Normal Range>)

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

<table>
<thead>
<tr>
<th>Concentration (U/ml)</th>
<th>OD 450/620 nm</th>
<th>CV % (Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.048</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>0.134</td>
<td>1.1</td>
</tr>
<tr>
<td>10</td>
<td>0.280</td>
<td>2.4</td>
</tr>
<tr>
<td>30</td>
<td>0.616</td>
<td>2.5</td>
</tr>
<tr>
<td>100</td>
<td>1.201</td>
<td>1.8</td>
</tr>
<tr>
<td>300</td>
<td>2.062</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Example of calculation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Replicate (OD)</th>
<th>Mean (OD)</th>
<th>Result (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 01</td>
<td>0.756/0.739</td>
<td>0.748</td>
<td>44.0</td>
</tr>
<tr>
<td>P 02</td>
<td>1.231/1.204</td>
<td>1.218</td>
<td>100.2</td>
</tr>
</tbody>
</table>

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

Do not use this example for interpreting patients results!

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

For qualitative interpretation read the optical density of the cut-off calibrator and the patient samples. Compare patient’s OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative: \[ \text{OD}_{\text{patient}} < 0.8 \times \text{OD}_{\text{cut-off}} \]

Equivocal: \[ 0.8 \times \text{OD}_{\text{cut-off}} \leq \text{OD}_{\text{patient}} \leq 1.2 \times \text{OD}_{\text{cut-off}} \]

Positive: \[ \text{OD}_{\text{patient}} > 1.2 \times \text{OD}_{\text{cut-off}} \]
9. Technical Data

Sample material: serum
Sample volume: 10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time: 90 minutes at 20-32°C/68-89.6°F
Calibration range: 0-300 U/ml
Analytical sensitivity: 1.0 U/ml
Storage: at 2-8°C/35-46°F use original vials, only
Number of determinations: 96 tests

10. Performance Data

10.1 Analytical sensitivity
Testing sample buffer 30 times on Rib-P gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and sensitivity
The microplate is coated with native human ribosomal proteins P0, P1 and P2. No crossreactivities to other autoantigens have been found. The frequency of anti-rib-P antibodies is 10-20% in randomly selected SLE patients. Anti-rib-P antibodies are detected more frequently in lupus patients with severe psychiatric manifestations.

10.3 Linearity
Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Dilution Factor</th>
<th>measured concentration (U/ml)</th>
<th>expected concentration (U/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 / 100</td>
<td>118.0</td>
<td>117.0</td>
<td>100.9</td>
</tr>
<tr>
<td></td>
<td>1 / 200</td>
<td>54.0</td>
<td>58.5</td>
<td>92.3</td>
</tr>
<tr>
<td></td>
<td>1 / 400</td>
<td>27.0</td>
<td>29.3</td>
<td>92.2</td>
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<td>1 / 800</td>
<td>14.0</td>
<td>14.6</td>
<td>95.9</td>
</tr>
<tr>
<td>2</td>
<td>1 / 100</td>
<td>16.4</td>
<td>15.0</td>
<td>109.0</td>
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<tr>
<td></td>
<td>1 / 200</td>
<td>7.0</td>
<td>7.5</td>
<td>93.3</td>
</tr>
<tr>
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<td>1 / 400</td>
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<td>3.8</td>
<td>102.6</td>
</tr>
<tr>
<td></td>
<td>1 / 800</td>
<td>2.0</td>
<td>1.9</td>
<td>105.3</td>
</tr>
</tbody>
</table>
10.4 Precision
To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (U/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94.3</td>
<td>9.3</td>
</tr>
<tr>
<td>2</td>
<td>11.7</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>8.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (U/ml)</th>
<th>CV (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>98.6</td>
<td>6.2</td>
</tr>
<tr>
<td>2</td>
<td>14.9</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>10.2</td>
<td>0.8</td>
</tr>
</tbody>
</table>

10.5 Calibration
Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11. Literature

1. **Bonfa E, Weissbach H, Brot N, Elkon KB.**
   *Ribosomal P protein autoantibodies.*

   *Anti-P antibodies are associated with psychiatric and focal cerebral disorders in patients with systemic lupus erythematosus.*

3. **Elkon K, Parnassa A, Foster CL (1985).**
   *Lupus autoantibodies target ribosomal P protein.*

   *Identification and chemical synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus.*
   Proc Natl Acad Sci USA 83: 7419-7423.

5. **MacConnel WP and Kaplan NO (1982).**
   *The activity of the acidic phosphoproteins from the 80S rat ribosomes.*
ANNEX A: Pipetting scheme

We suggest pipetting calibrators, controls and samples as follows:

For **quantitative interpretation** use calibrators to establish a standard curve.

For **qualitative interpretation** use cut-off calibrator.

### Pipetting Scheme

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<td>A</td>
<td>CalA</td>
<td>CalE</td>
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<td>NC</td>
<td>P2</td>
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<td>C</td>
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<td>P1</td>
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PC: positive control
NC: negative control
CC: Cut-off calibrator
P1: patient 1
P2: patient 2
P3: patient 3
**Annex B: Test Procedure**

1. **Samples (1:101) / Controls**
   - Add 100 µl
   - Incubate for 30 minutes
   - Wash 3 times with 300 µl

2. **CONJ**
   - Add 100 µl
   - Incubate for 30 minutes
   - Wash 3 times with 300 µl

3. **SUB / STOP**
   - Add 100 µl
   - Incubate for 30 minutes
   - Add 100 µl
   - Incubate for 5 minutes
   - Measure OD₄₅₀
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</table>

Assay/Test: ____________________       Incubation / Inkub.: 1._________min   Date/ Datum:________

Temperature/Temperatur:__________ °F _________ °C    2._________min

Signature/Unterschrift:.____________ 3._________min

Name:____________________________