



REF 4000

April 17<sup>th</sup>, 2008

# Anti-rP

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG autoantibodies to ribosomal phosphoproteins in human serum

<b>REF</b>	Catalogue number	<b>LOT</b>	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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## INTENDED USE

**Anti-rP is used for the quantitative determination of IgG autoantibodies to ribosomal phosphoproteins (rP) in patients with Systemic Lupus Erythematosus (SLE) in human serum.**

The diagnostic hallmark of SLE is the determination of antibodies to dsDNA detectable in up to 95 % of patients. In addition to nuclear antibodies in up to 20 % of SLE patients autoantibodies to rP can be found. Due to the ready correlation with disease activity these autoantibodies can be used for the follow-up.

Human 80S ribosomes are composed of two distinct subunits, a larger 60S and a smaller 40S subunit, containing RNA and more than 80 different polypeptides or proteins. rP located within the 60S subunit of human ribosomes form a pentamer (P1P2)<sub>2</sub>P0 and are linked to the GTPase domain of this subunit. Autoantibodies to rP target a highly conserved carboxyl-terminal epitope common to all of the three P proteins. This single linear epitope comprises 22 amino acids. The

Autoantibodies to rP are highly specific for SLE and correlate tightly with the activity of this systemic autoimmune disease. SLE patients exhibit these autoantibodies in addition to dsDNA and Sm autoantibodies being the main serological makers for SLE so far. rP autoantibodies have been observed especially in SLE patients with liver (23%) and kidney involvement (56%).

SLE patients with neuro-psychiatric disorders also seem to show rP autoantibodies more frequently. However, the follow-up of psychotic strokes by rP autoantibody levels is discussed controversially.

Elcon K, Parnassa A, Foster CL: Lupus autoantibodies target ribosomal P protein. J Exp Med 1985 162:459-471

Teh LS, Hay EM, Amos N, Black D, Huddy A, Creed F, Bernstein RM, Holt PJ, Williams BD: Anti-P antibodies are associated with psychiatric and focal cerebral disorders in patients with systemic lupus erythematosus. Br J Rheumatol 32: 287-290

## TEST PRINCIPLE

Anti-rP is an enzyme immunoassay for the quantitative determination of IgG autoantibodies to ribosomal phosphoproteins (rP) in human serum.

Autoantibodies of the diluted patient samples, controls, and standards react with rP (P0, P1, P2) immobilized on the solid phase of a microtiter plate. Anti-rP guarantees the specific binding of rP autoantibodies of the specimen under investigation by employing rP purified from an eucaryotic cell line. Following an incubation period of 30 min at room temperature (RT), unbound serum components are removed by a wash step.

The bound autoantibodies react specifically with anti-human-IgG-antibodies conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at RT. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. This enzyme reaction is stopped by dispensing an acidic solution into the wells after 30 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound.

The standard curve is established by plotting the concentrations of the antibodies of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

## PATIENT SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

### Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

**Note:** *Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.*

The samples may be kept at 2 - 8 °C for up to two days. Long-term storage requires -20 °C.

## TEST COMPONENTS FOR 96 DETERMINATIONS

<b>A</b> <b>Ag</b> <b>96</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells (total 96 individual wells) coated with purified rP P0, P1 und P2	1 vacuum sealed with desiccant
<b>B</b> <b>BUF</b> <b>WASH</b>	<b>Concentrated wash buffer</b> for 1000 ml solution <b>50x</b>	20 ml concentrate capped white
<b>C</b> <b>DIL</b>	<b>Concentrated sample diluent</b> for 100 ml solution <b>5x</b>	20 ml concentrate capped white
<b>D</b> <b>CONJ</b>	<b>Conjugate</b> containing anti-human-IgG coupled with HRP	15 ml ready for use capped blue
<b>E</b> <b>SOLN</b> <b>TMB</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped black
<b>F</b> <b>HCl</b>	<b>Stop solution</b> 1.0 M HCl <b>1.0 M</b>	15 ml ready for use capped white
<b>1-6</b> <b>CAL</b>	<b>anti-rP standards</b> (diluted human serum) Conc.: 0, 3, 10, 30, 100, 300 U/ml	1.5 ml each ready for use
<b>P</b> <b>CONTROL</b>	<b>Positive Control</b> (diluted human serum) conc.: see leaflet enclosed	1.5 ml ready for use <b>+</b>
<b>N</b> <b>CONTROL</b>	<b>Negative Control</b> (diluted human serum) conc.: see leaflet enclosed	1.5 ml ready for use <b>-</b>

### Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

## Size and storage

Anti-rP has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-rP have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

### Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 50 times with de-ionized or distilled water. For example, dilute 1 ml of the concentrate with 49 ml of distilled water per strip. The wash solution prepared is stable at 2 - 8 °C up to 30 days.

Prepare a sufficient amount of sample diluent by diluting the concentrated diluent 5 times with de-ionized or distilled water. For example, dilute 10 ml of the concentrate with 40 ml of distilled water. The sample diluent prepared is stable at 2 - 8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

## ASSAY PROCEDURE

- Dilute patient sera with sample diluent (made of C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (made of C).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense  
**100 µl** standards (1 - 6)  
**100 µl** positive and negative control  
**100 µl** diluted patient samples into the respective wells.
3. Incubate **30 min** at room temperature (18-25°C).
4. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Incubate **30 min** at room temperature (18-25°C).
7. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **30 min protected from light** at room temperature (18-25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

## DATA PROCESSING

### We recommend lin / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the standards 1 - 6 on the ordinate, y-axis, versus their respective anti-rP-concentrations on the abscissa, x-axis, (lin. scale).

Anti-rP concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

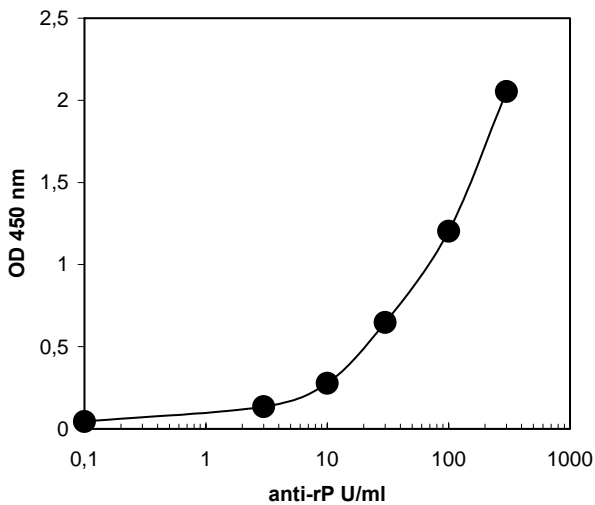
Anti-rP may be used also with Computer Assisted Analysis using software able to plot log/lin curves.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

### Example of typical assay results

well	OD (a)	OD (b)	OD (mean)	U/ml
Standard 1	0,043	0,047	0,045	0
Standard 2	0,132	0,138	0,135	3
Standard 3	0,285	0,271	0,278	10
Standard 4	0,619	0,675	0,647	30
Standard 5	1,190	1,216	1,203	100
Standard 6	2,079	2,029	2,054	300
patient 1	0,768	0,728	0,748	44

### STANDARD CURVE



### Test validity

The test run is valid if:

- the mean OD of the standard 1 is  $\leq 0.15$
- the mean OD of the standard 6 is  $\geq 1.3$

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

## REFERENCE VALUE

Anti-rP	
positive	$> 15$ U/ml
negative	$\leq 15$ U/ml

It is recommended that each laboratory establishes its own normal and pathological reference ranges for rP autoantibody levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

### Limitations of Method

Healthy individuals should be tested negative by the Anti-rP. However, rP autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

## CHARACTERISTIC ASSAY DATA

### Calibration

Due to the lack of an international reference material the Anti-rP is calibrated in arbitrary units (U/ml).

### Linearity

Dilutions of selected positive specimen in anti-ribosomal phosphoprotein free human serum are determined according to their expected theoretical values with Anti-rP.

### Sensitivity

The analytical sensitivity of the Anti-rP is 1 U/ml.

### Specificity

No cross reactivity to other autoantigens have been found.

### Precision

Intraassay		Interassay	
mean (U/ml)	CV %	mean (U/ml)	CV %
8.3	0.8	10.2	0.9
11.7	0.7	14.9	1.4
94.3	9.3	98.6	10.2

