

QUANTA Lite™ Ribosome P ELISA 708600

For *In Vitro* Diagnostic Use

CLIA Complexity: High

Intended Use

QUANTA Lite™ Ribosome P is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of Ribosome P antibodies in human serum. The presence of Ribosome P antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and other related connective tissue diseases.

Summary and Explanation of the test

Antinuclear antibodies (ANA) are found in a wide variety of connective tissue diseases and as such serve as a sensitive screening assay.¹ While ANA testing is an excellent screening test for SLE (a negative result virtually rules out active SLE)² it is by no means a specific test. A variety of specific follow-up tests are generally run on ANA positive specimens to determine the "autoantibody profile."

Autoantibodies reacting with cytoplasmic ribosomes are highly specific for SLE and as with antibodies to Sm and dsDNA are considered marker antibodies.³ By immunofluorescence using HEp-2 cell substrate, ribosome antibodies produce a finely granular cytoplasmic fluorescence.⁴ These antibodies are found in approximately 12% of patients with SLE^{5,6} and in 90% of patients with lupus psychosis.⁷ In most patients with lupus psychosis and ribosome antibodies, titers were found to increase more than five fold during and before active phases of the disease.⁷

Anti ribosome-P are directed to 3 phosphoproteins, P₀, P₁, and P₂, which are located on the larger 60S' subunit of eukaryotic ribosomes. Recently the ribosomal antigen has been very well characterized and studies have been published using either recombinant ribosome P⁸ or a synthetic carboxyl-terminal 22 amino acid peptide.^{9,10} This linear determinant peptide is common to and is the major epitope of the 3 ribosome phosphoproteins.¹⁰

Published studies using the synthetic 22 amino acid C-terminal peptide in an ELISA method show the test to perform well in the diagnosis of neurological lupus^{9,10} and to exhibit none of the conflicting results seen with recombinant ribosomal P¹¹ possibly due to presence of contaminating bacterial products within the antigen. A variety of methods including Ouchterlony double diffusion, RIA and mainly indirect immunofluorescence have been used to detect antibodies to ribosome. The immunofluorescence technique can give false negative results due to presence of interfering antibodies and false positive results due to reactivity with other nonribosome cytoplasmic antigens. The using a highly defined synthetic peptide as antigen yields in a sensitive, specific and reproducible assay. The ELISA technique employed in this test is sensitive, specific and objective. It can be conveniently used to test both large and small numbers of samples.

Principles of the Procedure

Synthetic Ribosome P antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any Ribosome P antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

Reagents

1. Polystyrene microwell ELISA plate coated with a purified Ribosome P antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to Ribosome P, prediluted, 1.2mL
3. Ribosome P ELISA Low Positive, 1 vial of buffer containing preservative and human serum antibodies to Ribosome P, prediluted, 1.2mL
4. Ribosome P ELISA High Positive, 1 vial of buffer containing preservative and human serum antibodies to Ribosome P, prediluted, 1.2mL
5. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
6. HRP Wash Concentrate, 1 vial of 40x concentrate - colored red containing Tris-buffered saline and Tween 20, 25mL. Refer to the Methods Section for dilution instructions.
7. HRP IgG Conjugate, (goat), anti-human IgG, 1 vial – colored blue containing buffer, protein stabilizers and preservative, 10mL
8. TMB Chromogen, 1 vial containing stabilizers, 10mL
9. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

Warnings

1. **WARNING:** This product contains a chemical (0.02% chloramphenicol) in the sample diluent, controls, and conjugate known to the State of California to cause cancer.

2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Ribosome P ELISA Low Positive, Ribosome P ELISA High Positive and ELISA Negative Control should be handled in the same manner as potentially infectious material.¹²
3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. Substitution of components other than those provided in this system may lead to inconsistent results.
3. Incomplete or inefficient washing and insufficient liquid removal from the ELISA well strips will cause poor precision and/or high background.
4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
5. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and liquid removal from the wells of the ELISA strips, the photometer used to measure the results, and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
6. Strict adherence to the protocol is recommended.
7. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
8. Unacceptably low absorbencies may be observed following **two** or more uses from a single bottle of HRP conjugate over a period of time. It is important to follow all recommended HRP conjugate handling procedures to prevent this occurrence.
9. Chemical contamination of the HRP conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol or detergent will cause degradation of the HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.

Storage Conditions

1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.
3. Diluted wash buffer is stable for 1 week at 2-8°C.

Specimen Collection

This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Following collection, the serum should be separated from the clot. NCCLS Document H18-A2 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

Procedure

Materials provided

- 1 Ribosome P ELISA microwell plate (12-1 x 8 wells), with holder
- 1 1.2mL prediluted ELISA Negative Control
- 1 1.2mL prediluted Ribosome P ELISA Low Positive
- 1 1.2mL prediluted Ribosome P ELISA High Positive
- 1 50mL HRP Sample Diluent

- 1 25mL HRP Wash Concentrate, 40x concentrate
- 1 10mL HRP IgG Conjugate, (goat), anti-human IgG
- 1 10mL TMB Chromogen
- 1 10mL HRP Stop Solution, 0.344M Sulfuric Acid

Additional Materials Required But Not Provided

Micropipets to deliver 5, 100, 200-300 and 500µL

Disposable micropipet tips

Test tubes for patient sample dilutions, 4mL volume

Distilled or deionized water

1L container for diluted HRP Wash Concentrate

Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

Method

Before you start

1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.
3. Prepare a 1:101 dilution of each patient sample by adding 5µL of sample to 500µL of HRP Sample Diluent. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the Ribosome P ELISA Low Positive, Ribosome P ELISA High Positive and ELISA Negative Control.
4. Determination of the presence or absence of Ribosome P using arbitrary units requires two wells for each of the three controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.

Assay procedure

1. **ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE (20-26°C) PRIOR TO BEGINNING THE ASSAY.** Place the required number of microwells/strips in the holder. **Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor.**
2. Add 100µL of the **prediluted** Ribosome P ELISA Low Positive, the Ribosome P ELISA High Positive, the ELISA Negative Control and the diluted patient samples to the wells. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.
3. Wash step: Thoroughly aspirate the contents of each well. Add 200-300µL of the **diluted** HRP Wash buffer to all wells then aspirate. Repeat this sequence twice more for a total of three washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well after each washing step. Maintain the same sequence for the aspiration as was used for the sample addition.
4. Add 100µL of the HRP IgG Conjugate to each well. Conjugate should be removed from the bottles using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. **TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.** Incubate the wells for 30 minutes as in step 2.
5. Wash step: Repeat step 3.
6. Add 100µL of TMB Chromogen to each well and incubate **in the dark** for 30 minutes at room temperature.
7. Add 100µL of HRP Stop Solution to each well. Maintain the same sequence and timing of HRP Stop Solution addition as was used for the TMB Chromogen. Gently tap the plate with a finger to thoroughly mix the wells.
8. Read the absorbance (OD) of each well at 450nm within one hour of stopping the reaction. If bichromatic measurements are desired, 620nm can be used as a reference wavelength.

Quality Control

1. The Ribosome P ELISA Low Positive, the Ribosome P ELISA High Positive and the ELISA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the Ribosome P ELISA Low Positive, the Ribosome P ELISA High Positive and the ELISA Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at $\leq -20^{\circ}\text{C}$.
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated.
 - a. The absorbance of the prediluted Ribosome P ELISA High Positive must be greater than the absorbance of the prediluted Ribosome P ELISA Low Positive, which must be greater than the absorbance of the prediluted ELISA Negative Control.

- b. The prediluted Ribosome P ELISA High Positive must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
- c. The Ribosome P ELISA Low Positive absorbance must be more than twice the ELISA Negative Control or over 0.25.
- d. The ELISA Negative Control and Ribosome P ELISA High Positive are intended to monitor for substantial reagent failure. The Ribosome P ELISA High Positive will not ensure precision at the assay cutoff.
- e. The user should refer to NCCLS Document C24-A for additional guidance on appropriate QC practices.

Calculation of Results

The average OD for each set of duplicates is first determined. The reactivity for each sample can then be calculated by dividing the average OD of the sample by the average OD of the Ribosome P ELISA Low Positive. The result is multiplied by the number of units assigned to the Ribosome P ELISA Low Positive found on the label.

$$\text{Sample Value (units)} = \frac{\text{Sample OD}}{\text{Ribosome P ELISA Low Positive OD}} \times \text{Ribosome P ELISA Low Positive (units)}$$

Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity). If a more accurate quantitation of patient antibody is required, serial dilutions of the patient sample should be run and the last dilution to measure positive in the assay should be reported as the patient's antibody titer.

Interpretation of Results

The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The values shown below are suggested values only. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

The sample can then be classified as negative, weak positive, moderate positive or strong positive according to the table below.

	Units
Negative	<20
Weak Positive	20 – 39
Moderate Positive	40 – 80
Strong Positive	>80

1. A positive result indicates the presence of Ribosome P antibodies and suggests the possibility of Systemic Lupus Erythematosus or other related connective tissue diseases.
2. A negative result indicates no Ribosome P antibody or levels below the negative cut-off of the assay.
3. It is suggested that the results reported by the laboratory should include the statement: "The following results were obtained with the INOVA QUANTA Lite™ Ribosome P ELISA. Ribosome P values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgG levels cannot be correlated to an endpoint titer."

Limitations of the Procedure

1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. Not all SLE patients are positive for Ribosome P.
3. Not all SLE patients with neurological problems will be positive for ribosome P antibodies.
4. It has been reported that Ribosome P antibodies increase and decrease during flares and remissions and titers are affected by therapy.
5. Results of this assay should be used in conjunction with clinical findings and other serological tests.
6. The assay performance characteristics have not been established for matrices other than serum.

Expected Values

The ability of the QUANTA Lite™ Ribosome P ELISA to detect Ribosome P antibodies was evaluated by comparison to a commercially available indirect immunofluorescent test available from INOVA Diagnostics. Results of the immunofluorescent test were determined as positive if a fine granular cytoplasmic fluorescence was observed on the HEp-2 cell substrate and negative if no fluorescence was observed.

Normal Range

Eighty-seven normal samples were tested. The normal population was approximately 90% female and ages ranged from 18-69 years. All 87 samples were completely negative in the QUANTA Lite™ Ribosome P assay. The strongest sample had 10 units of activity with the majority of the others falling between 2 and 6 units. The positive cutoff is 20 units.

Relative Sensitivity and Specificity

Samples submitted to a reference lab were tested for ribosome antibodies by both the QUANTA Lite™ Ribosome P ELISA and by immunofluorescence on HEp-2 cells. The results appear below.

		ELISA			
		+	-		
HEp-2	+	11	2*	Relative Sensitivity	84.6%
	-	0	20	Relative Specificity	100%
				Relative Efficiency	93.9%

*Both of these samples were western blot negative.

Clinical Sensitivity and Specificity

Patient Group	No.	No. Ribosome P pos. (%)
Normals	122	0
Non-ribosome cytoplasmic antibodies	56	0
SLE	75	15 (20.0%)
SLE (neurological symptoms)	38	10 (26.3%)
SLE (no neurological symptoms)	19	0
SLE (neurological symptoms, ribosome positive by HEp-2 and/or western blot)	57	57 (100%)

Cross-reactivity

A variety of high titered autoantibody sera were tested. These samples included the following specificities: Sm, RNP, SS-A, SS-B, Scl-70, Jo-1, smooth muscle (ASMA), mitochondria M-2 (AMA) as well as serine protease-3 (PR-3), myeloperoxidase (MPO) and gliadin. All of these samples were found to be negative in the QUANTA Lite™ Ribosome P ELISA. The strongest sample had a value of 6 units with the majority of the rest falling in the 2-3-unit range. The cutoff for this test is 20 units.

Precision and Reproducibility

The precision and reproducibility of the assay was measured by running six replicates each of negative, weak positive and strong positive samples in four separate assays. The mean of the strong positive was 113.7, the weak positive was 25.1 and the negative was 2.72. The standard deviation and coefficient of variation for each sample are summarized below.

	Negative		Strong Positive		Weak Positive	
	SD	CV	SD	CV	SD	CV
Overall	0.42	15.2%	5.09	4.5%	0.83	3.3%
Within Run	0.14	5.1%	1.84	1.6%	0.81	3.2%
Between Run	0.43	15.6%	5.40	4.7%	0.76	3.0%

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Manufactured By:

INOVA Diagnostics, Inc.
9900 Old Grove Road
San Diego, CA 92131
United States of America

Authorized Representative in the EU:

Medical Technology Promedt Consulting GmbH
Altenhofstrasse 80
D-66386 St. Ingbert, Germany
Tel.: +49-6894-581020
Fax.: +49-6894-581021
www.mt-procons.com

Technical Service
628600USA

888-545-9495
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