CE IVD 2°C 8°C

DR. FOOKE LABORATORIEN GmbH

Please read instructions for use carefully before starting the assay

Ro52 ELISA

ELISA for the semi-quantitative determination of anti-Ro52 antibodies in human serum or plasma

REF 25006 Σ 96 Determinations

BACKGROUND

Circulating IgG autoantibodies to intracellular structures especially to nuclear antigens represent a characteristic feature of systemic autoimmune diseases. One of those antigens, the Ro52 could recently be identified as a RING dependent E3 Ligase. Autoantibodies specific for Ro52 occur in patients with systemic lupus erythematosus (SLE), Sjögren Syndrome (SjS), mixed connective tissue disease (MCTD) and systemic sclerosis (SSc). The Ro52, together with Ro60 is often termed as SS-A antigen (**S**jögren **s**yndrome-**A** antigen). Anti-Ro52 antibodies are frequently accompanied by anti-La and anti-Jo-1 antibodies. Moreover, anti-Ro52 antibodies seem to play an important role for neonatal lupus and congenital heart block, especially those directed against certain Ro52 epitopes. Noteworthy, the prevalence of anti-Ro52 in PM and SSc is significantly higher than of anti-Ro60 antibodies. Therefore separate determination of anti-Ro52 and anti-Ro60 antibodies is required.

INTENDED USE

The Ro52 ELISA is intended for the semi-quantitative determination of antibodies specific for the Ro52 protein. The results of the Ro52 ELISA aid to the diagnosis of SLE, SjS, SSc and related autoimmune disorders and should be used as prognostic marker for the disease progression.

PRINCIPLE

The Ro52 microtiter ELISA plates are coated with recombinant Ro52 antigen. Initially, diluted patient samples (1:101), Controls and the Calibrator (undiluted) are applied to the microtiter wells. This leads to the binding of anti-Ro52 antibodies to the immobilized Ro52 antigen. After washing an anti-IgG horseradish peroxidase (HRP) conjugate is added which binds to the anti-Ro52 antibodies. Unbound material is removed by another washing cycle. Finally the binding of anti-Ro52 antibodies is visualized by incubation with TMB Substrate resulting in the development of a blue colour turning into yellow after stopping the reaction with Stop Solution. The optical density of the yellow colour is directly proportional to the amount of bound anti-Ro52 antibodies and can be measured spectrophotometrically at 450 nm. A Calibrator with a known concentration of anti-Ro52 antibodies is tested simultaneously with the samples. Semi-quantitative results can be determined by calculating the ratios from the OD value of the Calibrator and the samples.

KIT COMPONENTS

Microtiter strips, antigen coated	MICROWELL	12 strips x 8 wells
Anti-IgG HRP-Conjugate	CONJ HRP G	1 x 15 mL
Concentrated Washing Buffer	WASHBUF B 25x	1 x 50 mL
TMB Substrate	SUB TMB	1 x 15 mL
Stop Solution (0.5 M H_2SO_4)	STOP H_2SO_4	1 x 12 mL
Dilution Buffer	DILBUF B	1 x 60 mL
Calibrator	CAL	1 x 2 mL
Negative Control	CONTROL −	1 x 2 mL
Positive Control	CONTROL +	1 x 2 mL

MATERIAL NEEDED, BUT NOT PROVIDED WITH THE KIT

2-10 µL, 10-100 µL and 200-1000 µL pipettes, Multipette, pipette tips, vials for diluting the specimen, graduated glass cylinder, microplate-reader, covering foil, microplate-washer (optional).

SPECIMEN COLLECTION & PREPARATION

Either Serum or Plasma can be used in this test. No additives or preservatives are necessary to maintain the integrity of the specimen.
Specimens should be stored at 2-8°C and assayed within 48 hours after collection. If the assay cannot be performed within 48 hours or if the specimen has to be shipped, cap the specimen and keep it frozen. Repeated freezing and thawing should be avoided. Frozen specimens should be thawed at room temperature (RT, 20-25°C) and mixed thoroughly by gentle inversion before assaying. The use of haemolysed or lipemic specimens is not recommended.

PREPARATION OF REAGENTS

Allow all reagents to come to RT before use.
Unused microtiterstrips have to be resealed properly in the provided foil bag containing a desiccant.

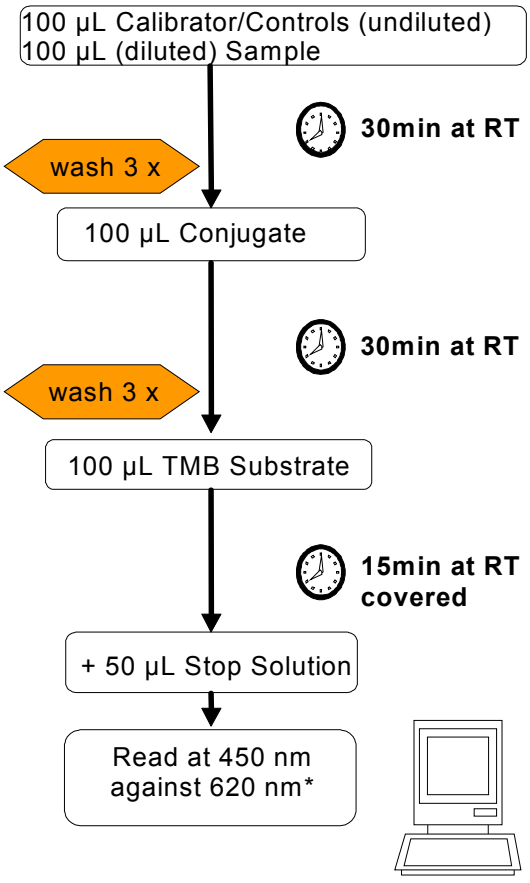
Dilution Buffer: ready to use
HRP Conjugate: ready to use
Substrate Solution: ready to use
Stop Solution: ready to use
Calibrator and Controls: ready to use

Concentrated Washing Buffer:
The Concentrated Washing Buffer has to be diluted 1:25 in aqua bidest. (Example: One strip requires 40 mL of Washing Buffer, therefore 1.6 mL Concentrated Washing Buffer have to be diluted to a final volume of 40 mL with aqua bidest.). The resulting Washing Buffer is stable for one week at RT.

ASSAY PROCEDURE

1. Create a pipetting scheme. It is a must to test the Calibrator in duplicate and this is highly recommended for Controls.
2. Dilute patient samples **1:101** in Dilution Buffer (10 µL serum + 1 mL Dilution Buffer for double determination / 5 µL serum + 0.5 mL Dilution Buffer for single determination).
3. Place the required coated wells into a frame. Properly reseal the aluminium bag with the remaining strips and desiccant.
4. Pipette 100 µL of Calibrators, Controls and diluted patient samples into the antigen coated wells according to the pipetting scheme.
5. Cover the plate and incubate for 30 min at RT.
6. Wash the plate manually or with an appropriate ELISA plate washer at least 3 times with minimally 300 µL per well. Remove residual liquid by dunking the microplate on a tissue.
7. Add 100 µL of anti-IgG HRP conjugate to all wells. Cover the plate and incubate for 30 min at room temperature.
8. Repeat washing procedure as described in step 6.
9. Add 100 µL of TMB Substrate to each well, cover the plate and incubate for 15 min at RT (TMB substrate is light sensitive).
10. Pipette 50 µL of Stop Solution in the same order as the substrate to each well. It is recommended to mix the solution in the wells by carefully knocking on the frame. Read OD at 450 nm (reference wave length 620 nm*) using an appropriate microplate reader and calculate the results of patient samples and controls as described on page 3.

TESTING SCHEME AI-LINE ELISA



* The measurement against the reference wave length from 620 nm is optional.

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CALCULATION OF RESULTS

Calculate the ratios between the OD value [Δ 450 nm – 620 nm] of each patient sample and the mean OD value [450 nm] of the Calibrator. Do the same for the controls. Multiply all obtained ratios by the conversion factor (F). This conversion factor is lot specific and is stated on the Quality Control Certificate. Resulting values are expressed as relative units (RU).

Calculation:

$$RU\ sample = \frac{OD\ Sample}{OD\ Calibrator} \times F$$

Example:

OD Calibrator = 1.9
OD Sample = 0.6
Conversionfactor F =10

$$RU\ sample = \frac{OD\ Sample}{OD\ Calibrator} \times 10$$

$$RU\ sample = \frac{0.6}{1.9} \times 10$$

RU sample = 3.2 RU

RESULT INTERPRETATION

For interpretation of the results use the following cut-off values.

< 1 RU	Negative
1-1.5 RU	Borderline
> 1.5 RU	Positive

The cut-off values were determined using disease controls and normal sera.

VALIDATION CRITERIA

The OD value of the Calibrator and the RU values of the Controls have to meet the ranges stated on the QC-Certificate. Otherwise, the test conditions should be verified and the test should probably be repeated.

REFERENCE RANGES

The average value of samples from apparently healthy controls was found at 0.3 RU (Standard Deviation 0.1 RU). Each laboratory should establish its own reference ranges.

MEASURING RANGE

0.1 up to 10 relative units (RU).

PRECISION

Variability and reproducibility was evaluated with three different positive sera. The Intra-assay variability for a quadruplicate measurement was below 7%. The Inter-assay variability, determined with duplicates taken from five different runs, was below 10%.

SPECIFICITY

The clinical specificity was found at 100% tested against healthy donors and unrelated disease controls.

SENSITIVITY

The analytical sensitivity in comparison to a validated FDA approved Reference system was found at 98% tested against healthy donors and unrelated disease controls. The clinical specificities for SjS and PM were found at 78.9% and 41.7%, respectively.

LITERATURE

1. Tan EM: **Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology.** *Adv Immunol* 1989, **44**:93-151.
2. Espinosa A, Zhou W, Ek M, Hedlund M, Brauner S, Popovic K, Horvath L, Wallerskog T, Oukka M, Nyberg F, Kuchroo VK, Wahren-Herlenius M: **The Sjogren's syndrome-associated autoantigen Ro52 is an E3 ligase that regulates proliferation and cell death.** *J Immunol* 2006, **176:**6277-6285.
3. Rutjes SA, Vree Egberts WT, Jongen P, Van Den Hoogen F, Pruijn GJ, Van Venrooij WJ: **Anti-Ro52 antibodies frequently co-occur with anti-Jo-1 antibodies in sera from patients with idiopathic inflammatory myopathy.** Clin Exp Immunol 1997, **109:**32-40.
4**.** Fritsch C, Hoebeke J, Dali H, Ricchiuti V, Isenberg DA, Meyer O, Muller S: **52-kDa Ro/SSA epitopes preferentially recognized by antibodies from mothers of children with neonatal lupus and congenital heart block**. *Arthritis Res Ther* 2005, 8:**R4.**
5. Schulte-Pelkum J, Fritzler M, Mahler M: **Latest update on the Ro/SS-A autoantibody system**. *Autoimmun Rev* 2009.

PRECAUTIONS FOR USERS

1. In compliance with article 1 paragraph 2b European directive 98/79/EC the use of *in-vitro* diagnostic medical devices is intended to secure suitability, performance and safety of the product by the manufacturer. Therefore the test procedure, information, precautions and warnings stated in the instructions for use have to be followed strictly. The kit has only to be used as described on page 1 (intended use).
2. The test must be performed according to this instruction, which contains all necessary information, precautions and warnings. The use of the test kit with analyzers and similar equipment has to be validated. Any change in design, composition of the test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes resulting in false results and other incidents. The manufacturer is not liable for any results obtained by visual analysis of patient samples.
3. The kit is intended for use by trained and qualified professionals carrying out research or diagnostic activities only. Pregnant women should not perform the test.
4. Laboratory equipment has to be maintained according to the manufacturer's instructions and must be tested for its correct function before use.
5. For *in-vitro* diagnostic use only. Use only once. Do not use components exceeding the expiry date. Do not combine reagents of other suppliers or kit components of different lots (unless specified on page 1) with this kit.
6. Do not use kit components when the package of the component is damaged. Please check all solutions prior to use for microbiological contamination. Cap vials tightly immediately after use to avoid evaporation and microbiological contamination. Do not interchange screw caps of the reagent vials.
7. The kit was evaluated for use at the temperatures specified in the Testing scheme (see page 2). Higher or lower temperatures may result in values not meeting the quality control ranges.
8. The washing procedure is absolutely important. Improper washing will cause erroneous results. It is recommended to use a multichannel pipette and an automated washer.
9. To avoid cross-contamination and false-positive results it is recommended to perform all pipetting steps properly. Use only clean pipette tips, dispensers and lab ware.
10. Test components based on human serum were tested using a CE marked method for the presence of antibodies against HIV 1 / HIV 2, Anti-HBc, and Anti-HCV as well as for hepatitis antigen HBsAg and were found to be negative. Nevertheless, material based on human serum should be handled as potentially infectious (BIOHAZARD).
11. Some kit components may contain bovine serum albumin, of which according to the manufacturer no infectious potential is known. Due to the eventual occurrence of undetectable infectious agents we recommend to handle any product of animal origin as potentially infectious.
12. The following safety rules should be followed with all reagents:
    - Do not get in eyes, on skin, or on clothing (P262). Do not breathe spray (P260). Pipetting should never be done by mouth, but with suitable pipetting devices.
    - IF SWALLOWED: rinse mouth. Do NOT induce vomiting (P301/330/331)
    - IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower (P303/361/353).
    - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing (P303/340).
    - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305/351/338)
    - Don't eat, drink or smoke while performing the test. Keep away from food, feed and beverage.
    - Wear protective gloves/protective clothing/eye protection (P280). Wash hands thoroughly after handling (P264) and care for your skin.
    - Material safety data sheet is available on request.
13. Stop Solution causes severe skin burns and eye damage (H314).
14. TMB in high concentrations may be potentially mutagenic. Due to the low concentration of TMB in this substrate solution a mutagenic effect can be ruled out, if it is properly used.
15. The preservatives (Bronidox, Thimerosal, Azid) are toxic to aquatic life, but their concentration is not hazardous to environment anymore. On disposal, flush large volumes of reagents with plenty of water. Thimerosal (WashBuf B) may cause damage to organs through prolonged or repeated exposure (H373).
16. Waste containing serum must be collected in separate containers containing an appropriate disinfectant in sufficient concentration. This material has to be treated according to national biohazard and safety guidelines or regulations.
17. We refer to the national regulations of medical devices regarding *in-vitro* diagnostic test kits.

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LOT	CE	IVD	8°C / 2°C		REF							
Lot- Number	European conformity	For *in-vitro* diagnostic use	Temperature Limit	Use before	Catalogue Number	Consult instructions for use	Refer accompanying documents	Do not use when package is damaged	Do not Re-use	Sufficient for <n> tests	Manu-factured by	Bio-hazard