Human Anti-SSA (Ro-60) ELISA
Cat No: K12-5458 Ver 1.0

Principle:
The method employs sandwich ELISA technique. SSA antigen are pre-coated onto microwells. Samples and Controls are pipetted into microwells and Human Anti-SSA (Ro-60) present in the sample are bound by the antigen. HRP Antigen Conjugate is added and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Human Anti-SSA (Ro-60) in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Intended Use:
This kit is used for the qualitative detection of Anti-SSA (Ro-60) in Human serum, plasma and other biological samples. The Kit is For Laboratory / Research Use Only.

Materials provided:
1. SSA antigen Microtiter Coated Plate (96 wells) – 1 no
2. Positive Control – 0.5 ml
3. Negative Control – 0.5 ml
4. HRP Conjugate – 6 ml
5. Wash Buffer (20X) - 25 ml
6. Sample Diluent – 6 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator
8. Timer.

Storage Information:

1. All reagents should be stored at 2ºC to 8ºC.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 30 minutes prior before use, bring all components to room temperature (18-25 ºC). Store all the components of the kit at its appropriate storage condition after use.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:
1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all samples in accordance with NCCLS regulations.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.
1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.

2. **Serum-** Coagulate the serum at room temperature (about 1 hours). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.
3. **Plasma-** Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20℃.
4. **Urine-** Collect urine in a sterile container, centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
5. **Cell Culture Supernatant-** Collect sample in a sterile container. Centrifuge for 20-mins at 2000-3000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2-7.4), if cell concentration is greater than 1 million/ml. Damage the cells by repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2000-3000 rpm. If precipitation appears, centrifuge again.
6. **Tissue Samples-** Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes and collect the supernatant carefully.

Note: Samples to be used within 5 days may be stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X);** dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water**.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Human Anti-SSA (Ro-60). High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Human Anti-SSA (Ro-60) present in the sample.
3. Avoid assay of Samples containing Sodium Azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Human Anti-SSA (Ro-60).
4. It is recommended that all Controls and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to poor sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. Bring the kit at room temperature before use. It is strongly recommended that all Controls and Samples be run in duplicates or triplicates.

2. Label the sample wells, Negative Control, Positive Control wells in duplicates.

3. Add **40 ul sample diluent and 10 ul sample** to the sample well.

4. **Add 50 ul Negative Control and 50 ul Positive Control** to respective wells.

5. Cover the plate with a sealer and incubate for 30 minutes at 37°C

6. Aspirate and wash plate 4 times with diluted Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

7. Add **50 ul HRP Conjugate** to each well, except blank well, gently tap the plate to ensure thorough mixing.

8. Cover the plate with a sealer and incubate for 30 minutes at 37°C

9. Aspirate and wash plate 4 times with diluted Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

10. Pipette **100 ul of TMB Substrate to each well.**

11. Cover the plate with a sealer and incubate for 10 minutes at 37°C. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

12. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

13. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Critical Value = Mean of Negative Control + 0.15

Validity of the test:

Test is valid if the following conditions are met, if not we recommend to re-test
Mean Absorbance of Negative Control ≤0.15;
Mean Absorbance of Positive Control ≥1.00;

Interpretation of Results

Negative Sample: if the sample OD value < Critical Value, the Human Anti-SSA (Ro-60) is Negative;
Positive Sample: if the sample OD value ≥ Critical Value, the Human Anti-SSA (Ro-60) is Positive.

Precautions:
Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Limitations:
1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. The reagent is a qualitative reagent, and cannot be used as a quantitative reagent.
3. The reagent is only used for the detection of human **serum, plasma and other biological samples**.

Performance Characteristics:
1. Negative Specificity: All results should be negative when detecting national negative quality control samples with ELISA kits of Anti-SSA (Ro-60).
2. Positive Specificity: All results should be positive when detecting national positive quality control samples with ELISA kits of Anti-SSA (Ro-60).
3. Unit of Detection: At least three sixth results should be positive when detecting Anti-SSA (Ro-60) national limit quality control samples with the ELISA kits of Anti-SSA (Ro-60), the matrix fluid should result in negative.
4. Precision: Repeat the test for 10 times with the quality control material, the CV should not over 15%.
5. Inter-assay: Repeat the test for the same sample with 3 batches test kit for 10 times respectively, the CV should not over 20%.
6. Specificity Analysis: Add 120 IU/ml rheumatoid factor, 1400 nmol/ml bilirubin, and 34 umol/ml triglyceride into the sample did not interfere with the test results. The sample contained 20 mg/ml hemoglobin cause the result in false positives. Hemolysis samples were not recommended; There is no cross reaction occurs with Hepatitis B, Dasanyang, Hepatitis A virus IgM antibody, human herpesvirus capsid antigen IgM antibody, rubella virus IgM antibody, Mycoplasma pneumonia IgM antibody, varicella zoster virus IgM antibody, Ro-60 antibody, antinuclear antibody and anti-mitochondrial antibody.
7. Hook Effect: There is not HOOK effect on testing for sample with high concentration Anti-SSA (Ro-60).

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