

Human Anti-Smooth Muscle Antibody (ASMA) ELISA

Cat No: K12-0588

Principle:

The Human Anti-Smooth Muscle Antibody (ASMA) is sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Anti-Smooth Muscle Antibody (ASMA) in samples. Standards or Samples are added to the microtiter well which is pre-coated with Human Smooth Muscle Antigen. Biotinylated Human Smooth Muscle Antigen is added to the microplate to form a complex. Subsequently Streptavidin-HRP conjugate is pipetted. After incubation and a washing step TMB Substrate A and B, are added. Blue color develops on incubation and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the Human Anti-Smooth Muscle Antibody (ASMA) in the samples is directly proportional to the yellow color developed in the wells.

Intended Use:

This Kit is used to assay the level of Human Anti-Smooth Muscle Antibody (ASMA) in human serum and plasma. The Kit is For Laboratory / Research Use Only.

Materials provided in the Kit:

1. Human Smooth Muscle Antigen Coated Microtiter Plate (96 wells) - 1 no
2. Human Anti-Smooth Muscle Antibody Standard (lyophilized, concentrated, 500 ng/ml) - 2 vials
3. Biotinylated Smooth Muscle Antigen (concentrated) - 120 ul
4. Streptavidin-HRP Conjugate (concentrated) - 120 ul
5. Sample Diluent - 20 ml
6. Biotin Antigen Dilution Buffer - 10 ml
7. HRP Conjugate Dilution Buffer - 10 ml
8. (25X) Wash Buffer - 30 ml
9. TMB Substrate - 10 ml
10. Stop Solution - 10 ml
11. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 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 2°C to 8°C.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. 20 minutes prior before use, bring all components to room temperature (18-25°C). 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.

Specimen Collection and Handling:

1. **Serum-** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000xg, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

2. **Plasma-** Collect plasma using EDTA-Na₂ or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000xg at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
3. **Tissue Homogenates-** As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-coating PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue Normal 9 ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer or ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disruptor or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000xg to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg,
4. **Cell Culture Supernatant-** Centrifuge supernatant for 20 minutes at 1000xg at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
5. **Cell Culture Lysate-** Commercial RIPA kits are recommended to follow the instructions provided. Generally 0.5 ml RIPA lysis buffer would be appropriate to 2x10⁶ cells, DNA must be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
6. **Other Biological Fluids-** Centrifuge samples for 20 minutes at 1000xg at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C (assay ≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided diluent, and several trials may be necessary. The test sample must be well mixed with the diluent. If samples are expected to have very high concentrations of the analyte, dilute the samples with PBS (pH 7.4) first and then further dilute with the Sample Diluent.

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 30 ml of (25X) Wash Buffer in 720 ml of DI water.
4. **Biotinylated Smooth Muscle Antigen Working Solution:** Prepare it within 1 hour before experiment. Calculate required total volume of the working solution: 0.1 ml / well x quantity of wells. (Allow 0.1-0.2 ml more than the total volume. Dilute the Biotinylated ASMA (concentrated) with Biotin Antigen Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 ul Biotinylated ASMA into 99 ul Biotin Antigen Dilution Buffer).
5. **Streptavidin:HRP Conjugate Working Solution:** Prepare it within 30 minutes before experiment. Calculate required total volume of the working solution: 0.1ml / well x quantity of wells. (Allow 0.1-0.2 ml more than the total volume. Dilute the Streptavidin:HRP Conjugate with Streptavidin:HRP Conjugate Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 ul of Streptavidin:HRP Conjugate into 99 ul of Streptavidin:HRP Conjugate Dilution Buffer).
6. **Standards Preparation:** Reconstitute original ASMA Standard with 1 ml of Sample Diluent. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the below table.

| Standard Concentration | Standard Vial | Dilution Particulars |
|------------------------|---------------|--|
| 500 ng/ml | Standard No.8 | Reconstitute with 1ml Sample Diluent |
| 250 ng/ml | Standard No.7 | 300 ul Standard No.8 + 300 ul Sample Diluent |
| 125 ng/ml | Standard No.6 | 300 ul Standard No.7 + 300 ul Sample Diluent |
| 62.5 ng/ml | Standard No.5 | 300 ul Standard No.6 + 300 ul Sample Diluent |
| 31.25 ng/ml | Standard No.4 | 300 ul Standard No.5 + 300 ul Sample Diluent |
| 15.625 ng/ml | Standard No.3 | 300 ul Standard No.4 + 300 ul Sample Diluent |
| 7.812 ng/ml | Standard No.2 | 300 ul Standard No.3 + 300 ul Sample Diluent |
| 0 ng/ml | Standard No.1 | 300 ul Sample Diluent only |

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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-Smooth Muscle Antibody (ASMA). High Dose Hook Effect is due to excess of antibody for very high concentrations of Human Anti-Smooth Muscle Antibody (ASMA) present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the Human Anti-Smooth Muscle Antibody (ASMA) concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
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-Smooth Muscle Antibody (ASMA).
4. It is recommended that all Standards 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 Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
2. Add **100 ul prepared Standards and diluted Samples** to respective standard wells.
3. Cover the plate with a sealer and incubate for 90 minutes at 37°C.
4. 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.
5. Pipette **100 ul Biotinylated Smooth Muscle Antigen Working Solution** to all wells.
6. Cover the plate with a sealer and incubate for 60 minutes at 37°C.
7. Aspirate and wash as per Step (4) above.
8. Pipette **100 ul Streptavidin:HRP Conjugate Working Solution** to all wells. Mix well.
9. Cover the plate with a sealer and incubate for 30 minutes at 37°C.
10. Aspirate and wash as per Step (4) above.
11. Pipette **90 ul TMB Substrate** in all the wells.
12. Incubate the plate at **37°C for 10 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
13. Pipette **50 ul of Stop Solution** to all wells. The wells should turn from blue to yellow in color.
14. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

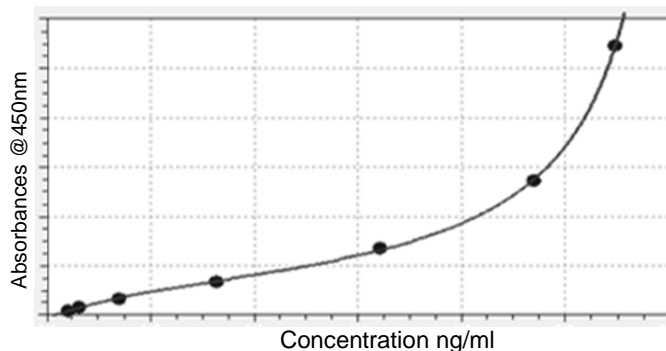
Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples. Use the Net Absorbance (Absorbance of Standard/Sample - Absorbance of Blank) to calculate the Mean Absorbances. Using standard graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis.

Draw the best fit curve through the standard points. To determine the unknown concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or a polynomial regression to the 2nd order is best recommended for automated results.

Typical Graph



Precautions:

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

Performance Characteristics:

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay and the dilutional linearity assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

Sensitivity:

Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to **4.688 ng/ml**.

Specificity:

This assay has high sensitivity and excellent specificity for detection of ASMA. No significant cross-reactivity or interference between ASMA and analogues was observed.

Recovery

Matrices listed below were spiked with certain level of ASMA and the recovery rates were calculated by comparing the measured value to the expected amount of ASMA in samples.

| Matrix | Recovery Range (%) | Average (%) |
|---------------------|--------------------|-------------|
| Serum(n=5) | 86-103 | 95 |
| EDTA Plasma(n=5) | 86-104 | 97 |
| Heparin Plasma(n=5) | 91-104 | 97 |

Assay Range:

7.812 ng/ml - 500 ng/ml

Precision:

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Linearity:

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of ASMA and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

| Sample | 1:2 | 1:4 | 1:8 |
|----------------------|---------|---------|--------|
| serum (n=5) | 87-105% | 90-105% | 85-99% |
| EDTA plasma (n=5) | 86-96% | 86-98% | 82-98% |
| heparin plasma (n=5) | 80-93% | 80-92% | 81-99% |

LIMITED WARRANTY

KinesisDx does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by KinesisDx, or against damages resulting from such non-KinesisDx made products or components. KinesisDx passes on to customer the warranty it received (if any) from the maker thereof of such non- KinesisDx made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by KinesisDx.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of KinesisDx shall be to repair or replace the defective product in the manner and for the period provided above. KinesisDx shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall KinesisDx be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of KinesisDx with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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ASSAY PROCEDURE

| | | | |
|----|--|--------|--------|
| 1 | Bring all reagents to room temperature before use. | | |
| 2 | Pipette Standards 1 - 8 Samples | 100 ul | 100 ul |
| 3 | Incubate 90 minutes (37°C) | | |
| 4 | Wash 1X Wash Buffer Decant, 4 x 300 ul | | |
| 5 | Pipette Biotinylated Smooth Muscle Antigen | 100 ul | 100 ul |
| 6 | Incubate 60 minutes (37°C) | | |
| 7 | Wash 1X Wash Buffer Decant, 4 x 300 ul | | |
| 8 | Pipette Streptavidin: HRP Conjugate | 100 ul | 100 ul |
| 9 | Incubate 30 minutes (37°C) | | |
| 10 | Wash 1X Wash Buffer Decant, 4 x 300 ul | | |
| 11 | TMB Substrate | 90 ul | 90 ul |
| 12 | Incubate in the dark 10 minutes (37°C) | | |
| 13 | Pipette Stop Solution | 50 ul | 50 ul |
| 14 | Measure 450 within 15 mins | | |