Version 06-05/24

User's Manual

Human ASMA(Anti Smooth Muscle Antibody) ELISA Kit

REF **DEIA-NS2310-1**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

In vitro quantitative determination of ASMA concentrations in serum, plasma, cell culture supernatant and other biological samples.

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Antigen was pre-coated onto the 96-well plate. The biotin conjugated antigen was used as the detection antigen. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antigen was added to bind with ASMA conjugated on coated antigen. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding acidic stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of target antigen in the sample is positively correlated with OD450 and can be calculated by plotting the standard curve.

Reagents And Materials Provided

No.	Item	Size(96T)	Storage Condition for Opened Kit
E001	ELISA Microplate(Dismountable)	8×12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C
E002	Lyophilized Standard	2vial	Put the rest standards into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 6 month at -20°C
E003	Biotin-labeled Antigen (Concentrated, 100X)	120ul	2-8°C (Avoid Direct Light)
E034	HRP-Streptavidin Conjugate(SABC, 100X)	120ul	
E024	TMB Substrate	10ml	
E039	Sample Dilution Buffer	20ml	2-8°C
E040	Antigen Dilution Buffer	10ml	
E049	SABC Dilution Buffer	10ml	
E026	Stop Solution	10ml	
E038	Wash Buffer(25X)	30ml	
E006	Plate Sealer	5 pieces	
E007	Product Description	1 copy	

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label. Please use pipette accurately measure and do proportional dilution.

Materials Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator (CO_2 incubator for cell culture is not recommenced.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips(calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Storage

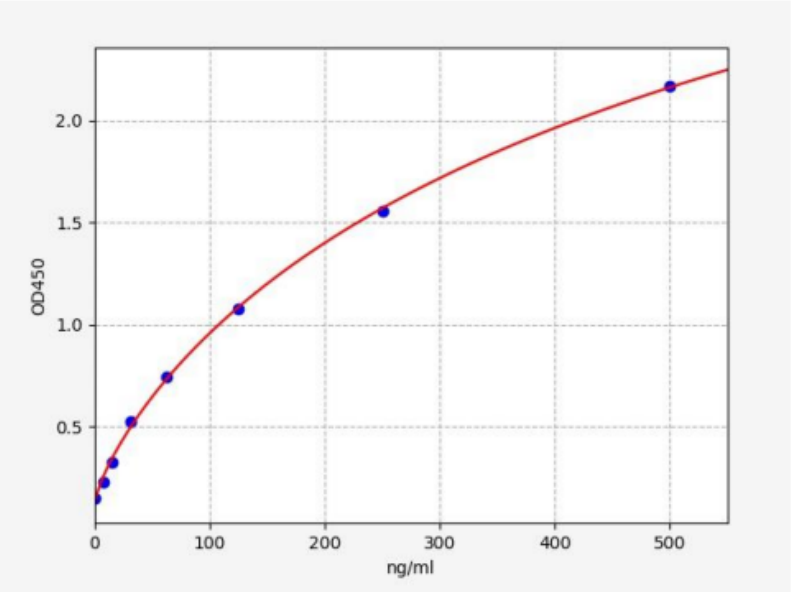
2-8°C (for sealed box), please do not freeze! See kit label for expiry date

Typical Standard Curve

This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C-25°C. TMB was balanced to 37°C before color development, and incubated at 37°C for 15 minutes in the dark after adding the enzyme label plate holes.)

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.105	0.11	0.107	0
7.812	0.177	0.184	0.181	0.073
15.625	0.207	0.216	0.211	0.104
31.25	0.318	0.331	0.324	0.217
62.5	0.532	0.554	0.543	0.436
125	0.902	0.939	0.92	0.813
250	1.278	1.33	1.304	1.197
500	2.2	2.29	2.245	2.138



Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	15.05	60.59	243.1	15.81	62.21	231.7
Standard deviation	0.82	2.88	9.94	0.76	3.21	11.26
CV(%)	5.46	4.75	4.09	4.83	5.16	4.86

Detection Range

7.813-500ng/ml

Sensitivity

4.688ng/ml

Specificity

Specifically recognize ASMA, no obvious cross reaction with other analogues

Linearity

Dilute the sample with a certain amount of ASMA at 1:2, 1:4 and 1:8 to get the recovery range.

Sample	1:2	1:4	1:8
Serum(n=5)	82-98%	81-91%	85-101%
EDTA Plasma(n=5)	84-101%	83-96%	85-105%
Heparin Plasma(n=5)	82-98%	86-95%	94-104%

Recovery

Add a certain amount of ASMA into the sample. Calculate the recovery by comparing the measured value with the expected amount of ASMA in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	86-100	95
EDTA Plasma(n=5)	90-105	97
Heparin Plasma(n=5)	85-105	94