Version 18-05/25

User's Manual

Human Soluble Elastin ELISA Kit

REF **DEIA-XY2207**

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.

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PRODUCT INFORMATION

Intended Use

For the quantitative determination of human soluble elastin concentrations in serum.

Principles of Testing

Human Soluble Elastin ELISA employs the quantitatively competitive enzyme immunoassay technique in which human soluble elastin present in samples compete with a fixed amount of biotinylated human elastin for sites on an antibody specific against human elastin. During the incubation, the standard and samples bound to the anti-human elastin IgG pre-coated onto the microplates. The biotinylated elastin competitively bound to antibody specific to elastin. Following a wash to remove any unbound standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the stop solution is added. The intensity of the color measured is in inverse proportion to the amount of human elastin bound in the initial step. The sample values are then read off the standard curve.

Human Soluble Elastin ELISA has been shown to accurately quantify the natural human elastin. Results obtained using natural human elastin showed dose response curves that were parallel to the standard curves obtained using the kit standards.

Reagents And Materials Provided

1. Assay Microplate: 1 plate, 96 well microplate pre-coated with a purified polyclonal anti Rabbit IgG
2. ELASTIN Standard: 1 vial, 2000 ng/vial of human ELASTIN in a buffered protein base with preservatives; lyophilized.
3. Antibody Solution Concentrated: 1 vial, 600 uL/vial, 10-fold concentrated of human ELASTIN biotinylated with preservatives; lyophilized.
4. Biotin Solution Concentrated: 1 vial, 600 uL/vial, 10-fold concentrated of human ELASTIN biotinylated with preservatives; lyophilized.
5. Positive Control: 1 vial, one vial of human ELASTIN, lyophilized (optional)
6. Streptavidin-HRP Conjugate: 1 vial, 120 ul/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP
7. Dilution Buffer: 1 bottle, 60mL of buffered protein based solution with preservatives. Ready to use.
8. HRP Diluent Solution: 1 bottle, 12mL of buffered protein based solution with preservatives. Ready to use.
9. Wash Buffer: 1 bottle, 50 mL of 10-fold concentrated buffered surfactant, with preservative.
10. TMB Substrate Solution: 1 bottle, 11 mL of TMB substrate solution
11. Stop Solution: 1 bottle, 11 mL of contains 0.5 M HCl
12. Plate Sealer: 1 piece
13. Plastic Pouch: 1

Materials Required But Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.

- Microplate shaker (250-300rpm).

- Pipettes and pipette tips.

- Deionized or distilled water.

- Squirt bottle, manifold dispenser, or automated microplate washer.

- 100 mL and 500 mL graduated cylinders.

Storage

Unopened Kit: Store at 2 - 8°C for up to 12 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Biotin Solution and SHOULD BE STORED at -20°C or - 70°C for up to one month. Reconstituted Biotin Solution (300 ul) CAN NOT BE STORED at 2-8°C. Streptavidin-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

Specimen Collection And Preparation

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples DO NOT require dilution. However, if the ELASTIN levels in samples are over 400ng/mL, a 2~4-fold or higher dilution would be required. A suggested 2-fold dilution is 60 uL sample + 60 uL Dilution Buffer. A suggested 4-fold dilution is 30 uL sample + 90 uL Dilution Buffer. Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.

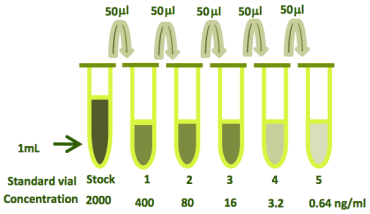
Reagent Preparation

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

Human ELASTIN Standard - Refer to vial label for reconstitution volume. Reconstitute the Human ELASTIN standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 uL of Dilution Buffer into tubes #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 ng/mL standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 mL	2000 ng/ml
# 1	50µl of stock	200µl	400 ng/ml
# 2	50µl of 1	200µl	80 ng/ml
# 3	50µl of 2	200µl	16 ng/ml
# 4	50µl of 3	200µl	3.2 ng/ml
# 5	50µl of 4	200µl	0.64 ng/ml



Antibody Solution - Reconstitute the Antibody Solution Concentrate with 600 ul of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 5.4 mL of Dilution Buffer to prepare 1x Antibody Solution.

Biotin Solution - Reconstitute the Biotin Solution Concentrate with 600 ul of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 5.4 mL of Dilution Buffer to prepare 1x Biotin Solution. 1x Biotin Solution should be prepared and used immediately. If you need repeat this assay next day, the 1x Biotin Solution should be stored at -20 ~ -70°C.

Streptavidin-HRP Conjugate - Transfer 120 ul of 100-fold concentrated Streptavidin-HRP Conjugate stock solution to 11.88 mL of HRP Diluent Solution to prepare working solution. Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

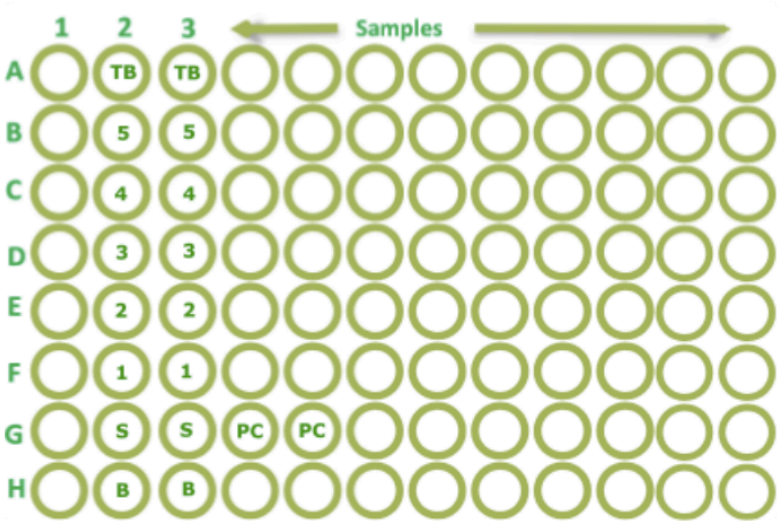
Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer. Note: Positive Control should be prepared and used immediately.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Leave well H2 and H3 as Blank. DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.
4. Set A2 and A3 as total binding (T). Add 50 ul per well of Dilution Buffer.
5. Add 50 ul per well of standard solution from #5 to S (reverse order of serial dilution) to the appropriate wells (B2, B3 to G2, G3). Add 50 ul per well of Positive Control into wells G4 and G5. Add 50 ul per well of samples into appropriate wells. Cover or seal the plate and incubate on microplate shaker (250-300rpm) at room temperature for 2 hours. Note: DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.
6. Add 50 ul per well of 1x Biotin Solution into total binding, standard, PC and samples wells. Cover or seal the plate and incubate at room temperature for 2 hours. Note: DO NOT ADD Biotin Solution to Blank wells.

7. Aspirate wells and wash 4 times with 300 ul of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
8. Add 100 uL of Streptavidin-HRP Conjugate working solution to each well. Incubate on microplate shaker for 60 minutes at room temperature. Protect from light.
9. Aspirate and wash as step 7.
10. Add 100 uL of Substrate Solution to each well. Incubate for 18-22 minutes at room temperature. Protect from light.
11. Add 100 uL of Stop Solution to each well. The color in the wells should change from blue to yellow. It is recommended to add the stop solution when the Total Binding or the lowest standard has developed a dark blue color.
12. Determine the optical density of each well within 15 minutes using a micro-plate reader set to 450 nm.



Calculation

Average the duplicate readings for each standard, positive control, and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

LAYOUT	STANDARD CONC. (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0	0 (0.062)
Stock STD	2000	0.084
STD1	400	0.243
STD2	80	0.528
STD3	16	0.781
STD4	3.2	0.892
STD5	0.64	0.965
Total Binding	0	1.085

Precision

Intra-assay Precision: 4-6%

Inter-assay Precision: 8-10%

Detection Range

3.2-400 ng/ml

Sensitivity

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human ELASTIN was 0.128 ng/mL

Specificity

Proteins Cross-reactivity

Human ELASTIN 100%

Human Periostin 0

Human SPARC 0

Linearity

To assess the linearity of the assay, the pooled research human serum samples in each matrix were diluted with Dilution Buffer and then assayed.

Sample type	Dilution Factor	Assayed (ng/ml)	Final (ng/ml)	Recovery (%)
Human serum	1	92.693	92.693	100
Human serum	2	49.552	99.104	107
Human serum	4	25.850	103.400	116

Precautions

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

Limitations

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ Some vials contain small quantities of material, therefore centrifuge before use.