HUMAN SOLUBLE ELASTIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE ELASTIN CONCENTRATIONS IN SERUM AND EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA Name	**Human SOLUBLE ELASTIN ELISA**
Catalog No.	SK00806-01
Lot No.	
Formulation	96 T
Standard range	0.64 – 2000 ng/mL
Dynamic Range	0.64 – 2000 ng/mL
Sensitivity	0.6 ng/mL
Sample Volume	120 or 60 µL per well per test
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA Plasma, Soluble Elastin
Specificity	Human Soluble Elastin
Calibration	Human soluble Elastin
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8°C for 6 months. Check page 2 -3 for more information.
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble Elastin ELISA kit contains the necessary components required for the quantitative measurement of natural human soluble Elastin from serum and plasma in a competitive EIA format.

This immunoassay contains human soluble Elastin pre-coated microplates and human soluble Elastin standard and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify natural soluble Elastin.

ASSAY OVERVIEW

The Human Soluble Elastin ELISA Kit employs the quantitatively competitive enzyme immunoassay technique in which soluble Elastin present in samples or Soluble Elastin Protein Standards were pre-incubated with Anti Human Soluble Elastin antibody, then pre-incubated antibody solutions compete with a fixed amount of Soluble Elastin proteins on the pre-coated microplate. Following a wash to remove any unbound primary antibody, standard and samples, the High Sensitivity Goat Anti Rabbit IgG HRP Conjugate was added to each well of Elastin Microplate. Following a final wash to remove any unbound enzyme, a TMB substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when stop solution is added. The intensity of the color measured is in inverse proportion to the amount of elastin immunoreactivity bound in the initial step. The higher the concentration of the elastin protein in solution is, the less the antibody bound to the plate will be. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL 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.

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

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Elastin-Microplate - 96 well microplate pre-coated with Human soluble Elastin protein.	**806-01-01**	**1 plate**
Elastin Standard – 2000 ng/vial of human soluble Elastin in a buffered protein base with preservative; lyophilized.	**806-01-02**	**1 vial**
Antibody Solution Concentrate – 700 µL/vial, 10-fold concentrate of polyclonal purified IgG against human soluble Elastin with preservative; lyophilized.	**806-01-03**	**1 vial**
Positive Control – one vial of human soluble Elastin; lyophilized.	**806-01-04**	**1 vial**
High Sensitivity Anti Rabbit IgG (H+L) HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of High Sensitivity Anti Rabbit IgG conjugate to HRP with preservative.	**HSARIGHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution.	**DB108A**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 Bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 Bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 Bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 8 months. For longer storage up to 10 months, unopened Standard, Positive Control, Antibody Concentrate, Biotin Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20°C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 - 8°C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of measuring absorbance at 450 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.
- 1.5 ml or 0.5 ml of microcentrifuge vails
- 200 µL of 96 well PCR plate or 8 well PCR strip.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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.

OPTIONAL: 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 need to be diluted.

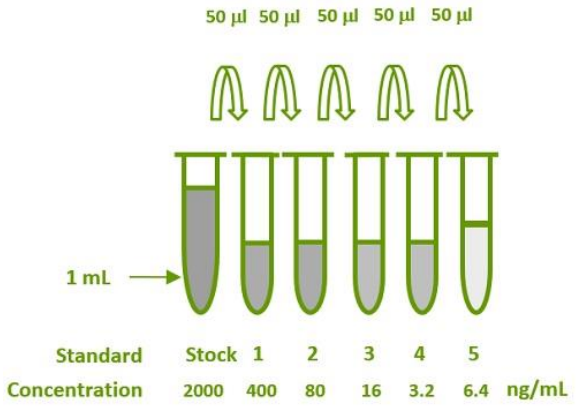
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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 1x Wash Buffer.

Elastin Standard - Reconstitute the soluble 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 µL of Dilution Buffer into tubes #2 to #6. Use the stock solution to produce a 5-fold dilution series (see below). Mix each tube thoroughly before the next transfer. The **2000 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	2000 ng/ml
# 1	80µl of stock	240µl	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



Serial Dilution of Soluble Elastin Standard

Positive Control - Reconstitute the Positive Control with refer to lot specific of Dilution Buffer.

Antibody Solution - Reconstitute the Antibody Solution Concentrate with 700 µL of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer 0.6 of 10-fold concentrated stock solution to 5.4 mL of Dilution Buffer to prepare **1x Antibody Solution**.

High Sensitivity Anti Rabbit IgG-HRP Conjugate - Transfer 120 µL of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution (DB08C)** to prepare working solution. **Protect from light. Note:** 1 x working solution of Anti rabbit IgG HRP should be freshly prepared and used within a few hours.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Pre-incubation 60 µL of standard, positive control, total binding (zero standard) and sample solutions with 60 µL of 1 x detection antibody in 1.5 ml of microcentrifuge vials or PCR 96 wells plate for 1 hour on Microplate shaker (300 rpm).
   Set 12 vials for standard, 2 vials for total binding and 2 vials for positive control. Set 38 or more vials for sample assay. 2.1. Add 60 µL of 1 x working solution of detection antibody to each vial or well. 2.2. Add 60 µL of each standard solution, positive control and 60 µL of sample solution into sample assay vials. Add 60 µL of Dilution Buffer in total binding serves as the zero standard (0 ng/mL).
3. After one hour for pre-incubation, transfer 100 µL of above pre-incubated solutions into each well of Elastin Microplate (806-01-01). Cover with plate sealer and incubate on microplate shaker (300rpm) at room temperature for 1 hours.
4. Leave two wells as Blank. ***DO NOT ADD ANY ANTIBODY OR SAMPLES INTO BLANK WELLS.***
5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µL of High Sensitivity Anti Rabbit IgG - HRP Conjugate working solution to each well, including blanks. Incubate on microplate shaker for 30 minutes at room temperature. **Protect from light.**
7. Aspirate and wash as step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate on microplate shaker for 13-18minutes at room temperature. **Protect from light**.
9. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing. It is recommended to add the stop solution when the Total Binding or the lowest standard has developed a dark blue color.
10. Determine the optical density of each well using a microplate reader set to 450 nm within 5 min.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, 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 DATA

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

Well	Average OD450nm	Standard (ng/mL)
Total Binding	2.298	0
Standard 5	2.288	0.64
Standard 4	2.118	3.2
Standard 3	1.666	16
Standard 2	0.859	80
Standard 1	0.433	400
Stock	0.144	2000

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble Elastin	100
Human Periostin	0
Human SPARC	0

Human Elastin recombinant derived from E. Coli may not be detected by this ELISA Kit.

LINEARITY

To assess the linearity of the assay pooled research human serum samples were diluted with Dilution Buffer and assayed.

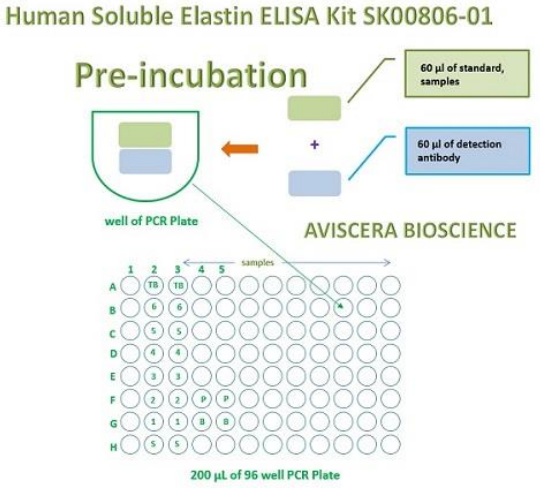
Dilution Factor	Assayed (ng/mL)	Final (ng/mL)	Recovery (%)
1x	101.000	101.000	100
2x	60.007	120.014	119
4x	34.252	137.007	136

To assess the linearity of the assay pooled research human EDTA plasma samples were diluted with Dilution Buffer and assayed.

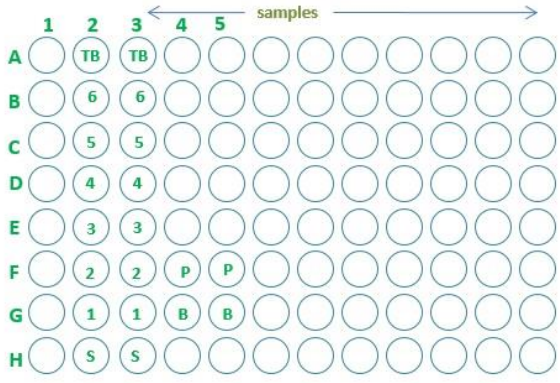
Dilution Factor	Assayed (ng/mL)	Final (ng/mL)	Recovery (%)
1x	141.151	141.151	100
2x	65.110	130.220	92.3
4x	33.690	134.761	95.5

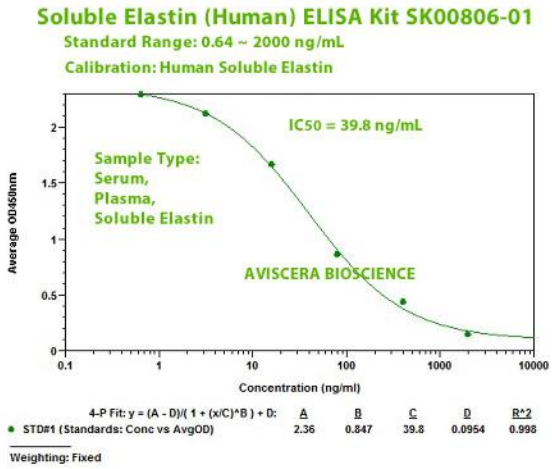
SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 60 µl of standard dilutions, samples, or positive control to the well. Add 60 µL of 1x Antibody solution to each pre-incubation vial or PCR well, except blanks. **Incubate 1 hour** on the microplate shaker at RT. **DO NOT WASH OR ASPIRATE. PROCEED TO NEXT STEP.**
↓
Transfer 100 µL of above pre-incubated Solution to each well used, except blanks. **Incubate 1 hour** on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Anti Rabbit IgG-HRP conjugate working solution to all wells, including blanks.
Incubate 30 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 13-18 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 5 min.



Elastin Assay Microplate




Soluble Elastin (Human) ELISA Kit SK00806-01
Standard Range: 0.64 ~ 2000 ng/mL
Calibration: Human Soluble Elastin
IC50 = 39.8 ng/mL
Sample Type:
Serum,
Plasma,
Soluble Elastin
AVISCERA BIOSCIENCE
Average OD450nm
Concentration (ng/ml)
4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:
A B C D R^2
STD#1 (Standards: Conc vs AvgOD) 2.36 0.847 39.8 0.0954 0.998
Weighting: Fixed