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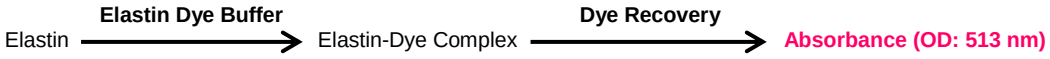
Elastin Assay Kit (Colorimetric)

02/21

(Catalog # K2077-100; 100 assays; Store at 4 ºC)

I. Introduction:

Elastin is a key protein of the extracellular matrix that provides resilience and elasticity to tissues and organs. It is highly elastic and is primarily present in extensible tissues including arteries, lung, ligaments and skin to resume their shape after stretching or contracting. Because of its critical role in normal development and function of vital organs, either an impairment of elastin synthesis or proteolytic degradation of the insoluble elastin fibers results in major clinical pathologies. Mutations in the Elastin gene may lead to diseases such as Williams-Beuren syndrome, Cutis laxa and Supravalvular aortic stenosis. Additionally, a number of skin diseases have been associated with abnormalities of elastin and marked fragmentation of elastin is observed in pulmonary emphysema. **BioVision's Elastin Assay Kit** is a quantitative dye-binding based method for the analysis of elastin levels in tissues and cultured cells. Due to its unique chemical composition and highly cross-linked nature, elastin is stable. But in this kit, elastin is denatured and solubilized under high heat and extreme pH conditions unlike other proteins. The isolated elastin then reacts with a dye to form a colored product, which is measured by absorbance at 513 nm. The colorimetric signal is directly proportional to the amount of elastin in samples. The assay is easy to perform, reliable and selective. It has a linear range of 5 µg-100 µg elastin per well.



II. Application:

- Estimation of elastin levels in various sample types.

III. Sample Types:

- Tissue lysate
- Cell lysate

IV. Kit Contents:

Components	K2077-100	Cap Code	Part Number
Elastin Dye Buffer	35 ml	Amber/NM	K2077-100-1
Precipitating Reagent	3 ml	NM	K2077-100-2
Dye Dissociation Buffer	35 ml	Amber/WM	K2077-100-3
Oxalic Acid (1 M)	35 ml	NM	K2077-100-4
Elastin Standard (10 mg/ml)	2 x 1 ml	Yellow	K2077-100-5

V. User Supplied Reagents and Equipment:

- Distilled Water
- PBS (optional)
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- Mechanical shaker
- Metal heating block with the thermostat set between 95 ºC and 100 ºC

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at 4 °C, protected from light. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay.

- **Elastin Dye Buffer and Dye Dissociation Buffer:** Store at 4 ºC, protected from light. Bring to room temperature (RT) before use.
- **Precipitating Reagent:** Store at 4 ºC. Keep on ice while in use.
- **Oxalic Acid (1 M):** Store at 4 ºC. Bring to RT before use. Prepare 0.25 M Oxalic Acid for the assay by diluting 1 M Oxalic Acid with dH_2O. ***Note:*** Oxalic Acid is corrosive, wear gloves when handling.
- **Elastin Standard (10 mg/ml):** Store at 4 ºC. Use within two months.

VII. Elastin Assay Protocol:

1. Sample Preparation:

a. For tissues: Rinse tissue samples with ice-cold dH_2O or PBS to remove any residual blood and mince using clean scissors. Transfer the minced tissue (0.2~5 mg) to a microfuge tube and add 1 ml of ice-cold 0.25 M Oxalic Acid. Homogenize the tissue and incubate on a metal heating block at 100 ºC for 1 hour. Vortex frequently and thoroughly. **For adherent cells:** Cultured cells can be removed using trypsin or any non-enzyme based cell dissociation solution. Wash the cells using PBS, centrifuge and aspirate the PBS. Resuspend the cell pellet in 0.5~1 ml of 0.25 M Oxalic acid per ~1 x 10^6 cells in a microfuge tube. Vortex thoroughly and incubate on a metal heating block at 100 ºC for 1 hour. **Notes:** a) Do not tighten the tube caps while heating. b) The extract volumes of 0.25 M Oxalic Acid added need to be recorded for calculating the elastin content.

b. Following 1 hour incubation, bring the tubes to RT. Centrifuge the homogenate at ≥ 10,000 x *g* and 4 °C for 15 min and transfer the acidic supernatant to a new microfuge tube. **The supernatant will be used for the assay**. Add 1 ml 0.25 M oxalic acid/or the same volume of 0.25 M oxalic acid added previously, to the **residual tissue/cell pellet** in the tubes and heat again at 100 ºC for 1 hour and vortex thoroughly. Repeat the previous steps up to 2~3 times to completely solubilize the tissue elastin. **The extract from the last round should not contain any elastin.**

c. Add 100 µl of supernatant into a separate 1.5 ml eppendorf tube labeled as **Sample**. Add 100 µl of 0.25 M Oxalic Acid into another 1.5 ml eppendorf tube labeled as **Background Control**.

Notes:

- For Unknown Samples, retain each of the oxalic acid extracts separately (acid-heat-centrifugation) and analyze each for elastin. The remaining extracts can also be used for measuring protein concentrations.
- We suggest testing different amounts of samples to ensure the readings of first time extracts are within the Standard Curve range.
- Record the Dilution Factor (D: see VII 5). For example, the dilution factor is 10 if 100 µl of sample was used for the assay after 1 ml of 0.25 M Oxalic Acid was used to treat the sample(s).

2. **Standard Curve Preparation:** Prepare 1 mg/ml Elastin solution by adding 40 µl of 10 mg/ml Elastin to 360 µl 0.25 M Oxalic Acid. Add 0, 20, 40, 60, 80, 100 µl of the 1 mg/ml Elastin working solution into a series of 1.5 ml eppendorf tubes to generate 0, 20, 40, 60, 80, 100 µg of Elastin/tube. Adjust the volume of all Standard tubes to 100 µl/tube with 0.25 M Oxalic Acid, mix well.
3. **Reaction Preparation:**

Notes:

- We suggest running Standard Curve with each assay.
- Bring the Elastin Dye Buffer and the Dye Dissociation Buffer to RT 30 min prior to the assay.

a. **Protein Precipitation:** Add 10 µl of ice cold Precipitating Reagent to each tube containing Sample, Background Control and Standard(s). Cap the tubes and vortex thoroughly to mix the contents and leave on ice for at least 1 hour (or 4 ºC overnight) to complete the precipitation of Elastin. Centrifuge the tubes at ≥ 10,000 x *g* and 4 °C for 15 min. Drain the liquid contents of the tubes into a beaker. Remove most of the remaining fluid from the tubes by firmly tapping the inverted tubes onto absorbent filter papers or other absorbent materials. **Note:** Complete removal of Precipitation Reagent is essential for accurate results.

b. **Elastin-Dye Complex:** For each vial containing Sample, Background Control and Standard(s), add 200 µl Elastin Dye Buffer. Cap the tubes and mix contents by vortexing. Place the tubes on a mechanical shaker at RT for 90 min, **protected from light** by wrapping the tubes in an aluminum foil. After 90 min incubation, centrifuge the tube at ≥ 10,000 x *g* and 4 °C for 15 min. Drain the liquid contents of the tubes into a beaker. Remove most of the remaining fluid from the tubes by FIRMLY tapping the inverted tubes onto absorbent filter papers or other absorbent materials. A cotton bud can be useful for removing any fluid droplets from the rim of the tube without touching the bottom. **Note:** Complete removal of Elastin Dye Buffer is essential for accurate results. There should not be more than 10 µl of fluid at the bottom of the tube.

c. **Dye Recovery:** There should be a brown residue in the Elastin Standard(s) and sample tubes after Elastin-dye binding. Add 200 µl Dye Dissociation Buffer to each tube containing Sample, Background Control and Standard(s). Cap the tubes and vortex at RT using a vortex mixer. Repeat the vortex from time to time to ensure that all the bound dye is into solution.

d. After 5-10 min, centrifuge the tubes and transfer 200 µl of the solution from each tube to wells of a 96 well clear plate with flat bottom.

4. **Measurement:** Measure the absorbance at OD 513 nm of all wells at RT in endpoint mode. **Note:** The signal is stable for at least 4 hours at RT in dark.
5. **Calculation:** Subtract the 0 Standard reading from all Standard readings and plot the Elastin Standard Curve and calculate the slope. Subtract the Background Control reading from its paired sample readings to get the corrected sample reading. Apply the corrected sample reading to the Elastin Standard Curve to get **B µg** of elastin in the well.

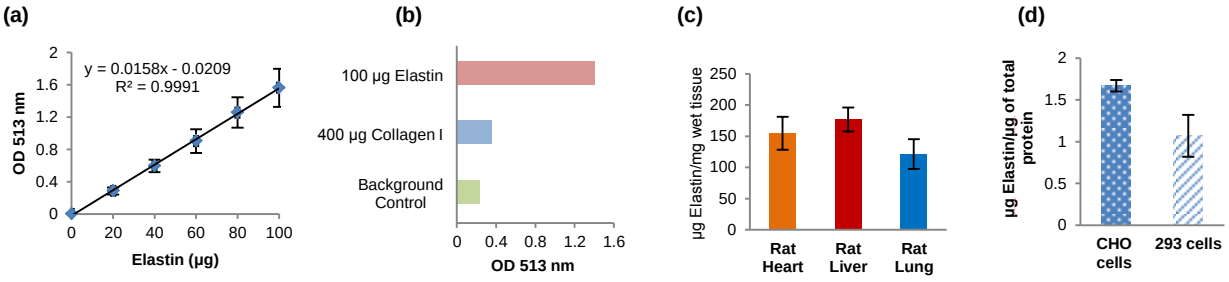
$$\textbf{Elastin Concentration in the sample well} = \frac{B}{W} * D = \textbf{µg Elastin/mg of sample}$$

Where, **B** is the amount of elastin, calculated from the Standard Curve (in µg)
W is the initial weight of sample (in mg)
D is the dilution factor

Or

$$\textbf{Elastin Concentration in the sample well} = \frac{B}{100 * P} = \textbf{µg Elastin/µg of total protein}$$

Where: **B** is the amount of Elastin, calculated from the Standard Curve (in µg)
100 is the volume of sample used for measurement (in µl)
P is the initial sample concentration (in µg protein/µl)



Figures: (a). Elastin Standard Curve. (b). Estimation of purified elastin and collagen I (in 0.25M Oxalic Acid and 100 ºC for 1 hour). (c) Estimation of elastin content in rat heart (2.58 mg wet tissue/1 ml 0.25M Oxalic Acid), rat liver (3.55 mg wet tissue/1ml 0.25M Oxalic Acid) and rat lung (2.88 mg wet tissue/1 ml 0.25M Oxalic Acid) samples. (d). Estimation of elastin content in CHO cells (10^6 cells/500 µl 0.25M Oxalic Acid) and HEK293 cells (10^6 cells/1 ml 0.25M Oxalic Acid). Data are mean ± SEM of 3 replicates, assayed according to the kit.

VIII. Related Products:

Total Collagen Assay Kit (K218 and K406)
Soluble Collagen Assay Kit (Fluorometric) (K532)
Hydroxyproline Assay Kit (K555 and K226)
Lysyl Oxidase Activity Assay Kit (Fluorometric) (K928)

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