AB308320 – Elastin Assay Kit (Colorimetric)

Estimation of elastin levels in various sample types
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
Elastin Dye Buffer	35 ml	4 °C
Precipitating Reagent	3 ml	4 °C
Dye Dissociation Buffer (Avoid light)	35 ml	4 °C
Oxalic Acid (1 M)	35 ml	4 °C
Elastin Standard (10 mg/ml)	2 x 1 ml	4 °C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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 °C and 100 °C

Reagent Preparation

Elastin Dye Buffer and Dye Dissociation Buffer: Store at 4 °C, protected from light. Bring to room temperature (RT) before use.

Precipitating Reagent: Store at 4 °C. Keep on ice while in use.

Oxalic Acid (1 M): Store at 4 °C. Bring to RT before use. Prepare 0.25 M Oxalic Acid for the assay by diluting 1 M Oxalic Acid with dH2O.
Δ Note: *Oxalic Acid is corrosive, wear gloves when handling.*

Elastin Standard (10 mg/ml): Store at 4 °C. Use within two months

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 °C 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 °C for 1 hour.

Δ Note:

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 °C 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 labelled as Sample. Add 100 µl of 0.25 M Oxalic Acid into another 1.5 ml Eppendorf tube labelled as Background Control.

Δ Note:

a) For Unknown Samples, retain each of the oxalic acid extracts separately (acid-heat-centrifugation) and analyse each for elastin. The remaining extracts can also be used for measuring protein concentrations.
b) We suggest testing different amounts of samples to ensure the readings of first-time extracts are within the Standard Curve range.
c) 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

Δ Note:

a) We suggest running Standard Curve with each assay.
b) 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 °C 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.

Δ 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.

Δ 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.

Measurement

Measure the absorbance at OD 513 nm of all wells at RT in endpoint mode.

Δ Note: *The signal is stable for at least 4 hours at RT in dark.*

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)

Technical Support

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