Fastin™ Product Manual

Product code:
F2000 (1x110 assay, Standard Kit)
F4000 (4x110 assay, Multipack)

This kit has been designed for Research Use Only

Always handle using Good Laboratory Practice

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PART A – General Information

Kit introduction

What is elastin?

The ability of cardiovascular, respiratory and musculoskeletal tissues to stretch and recoil repeatedly over an organism's lifetime owes much to the unique properties of the protein Elastin. Changes in elastin content and structure have long been of interest to those in the areas of aging and disease. In addition, elastin and elastin-based biomaterials are an area of key importance in the growing world of tissue engineering. Biocolor's Fastin elastin assay kit has therefore been developed as a user-friendly tool to enable analysis of this key connective tissue component.

Intended Applications

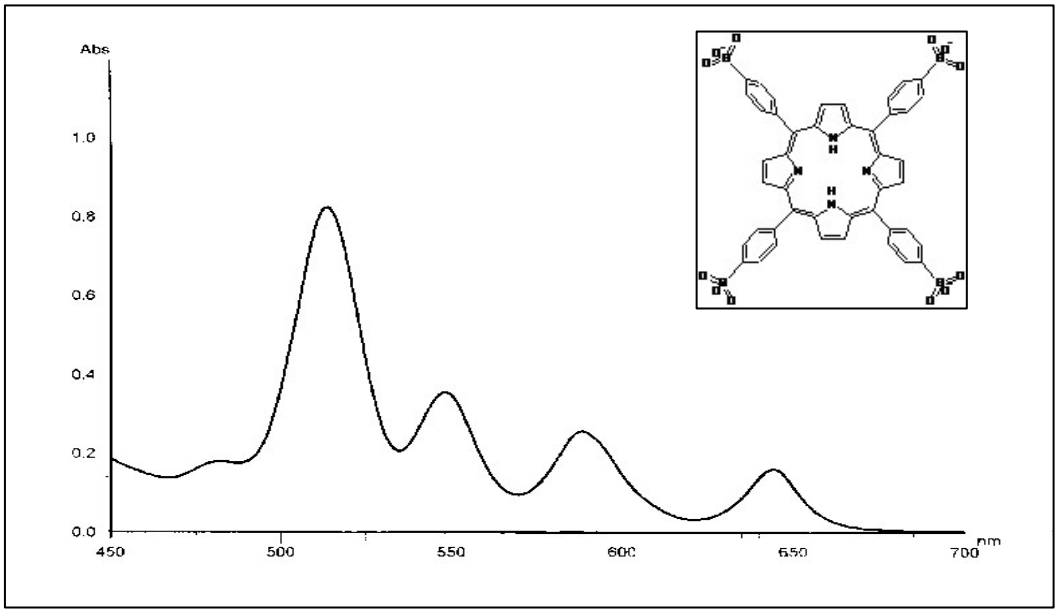
The Fastin Elastin Assay is a colorimetric, quantitative dye-binding method for the analysis of elastin extracted from *in-vivo* and *in-vitro* sources. A variety of elastin forms can be assayed, from the immature tropoelastin to mature 'insoluble' elastin fibres.

The dye label employed is *5,10,15,20-tetraphenyl-21H,23H-porphine tetra-sulfonate* (TPPS). Under assay conditions it binds the 'basic' and 'non-polar' amino acid sequences found in elastins. For the structural form/spectra of the dye see **Figure.1**.

NB: Applicability of the Fastin assay to different species

Customers are advised that the kit has been calibrated for samples of mammalian origin by virtue of the included mammalian-derived α-elastin Standard.
Analysis of elastin from other vertebrate species is possible, however the published limit of detection may be reduced due to variations in elastin sequence and dye-binding ability. For accurate quantification of samples of non-mammalian origin, customers may wish to replace the kit α-elastin Standard with a species-matched, purified preparation of elastin.

Figure.1 The visible absorbance spectrum and structural form of 'TPPS'.



How does the Fastin kit measure elastin?

The Fastin Dye reagent is formulated to bind specifically to elastin under assay conditions (Steps 1-3). This includes elastin that has been extracted from *in-vivo* and *in-vitro* tissue samples.

Step 1. Sequential incubation of samples containing soluble or solubilised elastins with Precipitating Reagent and Fastin Dye Reagent results in the formation of an insoluble elastin-dye complex.

Step 2. The insoluble, Dye-labelled elastin is isolated by centrifugation and the unbound dye removed. The Elastin-bound dye is then eluted and measured spectrophotometrically.

Step 3. The elastin content of unknown samples can be calculated by comparison against a calibration curve prepared using a reference standard comprising water-soluble elastin (supplied with the kit).

Table.1 Fastin Kit Specifications		
Limit of Detection	50 µg/ml	*In-vitro samples containing added animal serum will exhibit increased background absorbance and a reduced limit of detection*
Range	50 - 500µg/ml	
Detection Method	Colorimetric Detection (513nm) (Endpoint)	*Wavelengths between 510 – 515nm are optimal for analysis.*
Measurements per kit	110 in total	*Allows a maximum of 48 samples to be run in duplicate alongside a standard curve.*
Suitable samples	**In-vivo:** - Tissue extracts. NB: Mature (insoluble) elastin will first require conversion to water soluble α-elastin using a suitable extraction protocol (see *Part D*). **In-vitro:** - Elastin produced by cells during 2D/3D cell culture. - Liquid extracts containing soluble elastins, these include soluble tropoelastins, as well as insoluble elastins following extraction and conversion to soluble elastin peptides.	*In-vitro media samples containing added animal serum will exhibit increased background absorbance. When analysing such samples we strongly recommend using an appropriate negative control. (see p21 for more information).*
Sample Volume	100µl	*Larger volumes may be indicated in some sample preparation protocols*

Kit contents & Storage

The Fastin kit has a shelf life of 1-year when stored unopened at room temperature. The expiry date can be found on the Certificate of Analysis supplied with the kit.

Once opened, please follow the storage conditions in the **Table.2**.

Table.2 Fastin contents & storage conditions		
Component	**Volume supplied**	**Storage Recommendations (once kit is opened)**
Dye Reagent	110ml	4°C, away from light
α-elastin Standard (1mg/ml)	5ml	4°C
Precipitating Reagent	25ml	Room Temperature
Dye Dissociation Reagent	28ml	Room Temperature
Oxalic acid*	20ml	Room Temperature
Documentation**	QuickStart Guide / Manual / Certificate of Analysis	NA

**Oxalic acid will require dilution to 0.25M for sample extraction (see protocols for details).*

***Additional copies of the Assay Manual & QuickStart guide can be downloaded from Biocolor's website at https://www.biocolor.co.uk/assay-documentation*

What else is required to use this kit?

The following reagents or equipment may be required, depending on sample type used.

Microplate reader. *Used to measure dye released from elastin.*
Should be capable of 96-well microplate absorbance analysis at wavelengths between 510nm and 515nm (513nm is optimal).

Metal heating block/water bath. *Used to heat samples during elastin extraction.*
Should be capable of a holding a temperature of between 95 – 100°C. Otherwise a boiling-water resistant glass beaker on a hotplate with temperature maintained between 95 – 100°C can be used.

Vortexer and Mechanical Shaker. *Used to mix samples.*

Centrifuge. *Used to collect and retain precipitated/labelled elastin*
This should be fitted with a rotor head for 1.5 ml microcentrifuge tubes and capable of 10000 x *g*.

1.5ml, heat-resistant, screw-cap tubes (suitable for use at 100°C). *Used during the extraction of insoluble elastin from samples.*

PART B – The General Protocol

i.Setup of samples / standards / controls

We recommend assaying controls and samples in duplicate (as a minimum).

Setup Samples:
Ensure samples have been processed / extracted according to the selected preparation protocol.

Setup Controls:
It is always good practice to run assay controls. As a minimum, we would advise running a 'plate blank' comprising 150µl of Dye Dissociation Reagent, added to an empty microplate well during step 13). This can be subtracted from all other readings to correct for any background absorption from the microwell plate.

If using samples of *in-vitro* origin, please consult **Part D**, **section II** of the manual for further information on suitable controls.

Setup Reference Standards:
Prepare the dilutions of standards according to **Table.1**. For convenience these can be prepared directly within the 1.5ml green microcentrifuge tubes provided with the kit.

NB: *The 1M Oxalic Acid provided with the kit MUST BE diluted with diH_2O to a working concentration of 0.25M prior to preparation of standards.*

A suitable volume can be prepared by diluting the supplied 1.0M oxalic acid in the ratio 1:3. (i.e. to prepare a 10ml volume, add 2.5ml 1M oxalic acid stock to 7.5ml of diH2O).

Table 1. Preparation of assay Standards

Elastin Concentration (µg/ml)	Elastin Content (µg per tube)	Volume of Elastin reference standard (µl)	Volume of 0.25M oxalic acid (µl)
0	0	0	100
50	5.0	5	95
100	10.0	10	90
250	25.0	25	75
500	50.0	50	50

Please ensure that all tubes are suitably labelled before commencement of the assay.

ii.Fastin assay

NB: *Pre-chill the bottle of elastin Precipitating Reagent to 4°C before commencing the assay.*

1. Add 100µl of each prepared sample directly to a labelled 1.5ml microcentrifuge tube**.**

Precipitation of elastin

2. Add *Precipitating Reagent* to all tubes as follows:

    a. **Reference standards / Oxalic Acid extracted samples:**
    Add an equal (100 µl) volume of Elastin Precipitating Reagent to all tubes containing controls / samples.

OR

    b. **Culture medium samples:**
    Add 1.5x the volume (150 µl) of Elastin Precipitating Reagent to tubes that contain cell culture medium. *i.e. use 150 µl of Elastin Precipitating Reagent for every 100 µl of cell culture medium used*

3. Cap the tubes and briefly vortex to mix contents; then place in the fridge at 4°C for 15 minutes. *During this time, α-elastin will precipitate.*

4. Centrifuge tubes @ 13000 x *g* for 10 minutes. This causes precipitated elastin to form a pellet or coating on the inside tube surfaces. ***NB:*** *The pellet will be difficult to see until stained*

5. Drain tube's liquid contents into a beaker. While the tube is still inverted, remove most of the remaining fluid from the tube by tapping the inverted tube onto a single thickness absorbent paper towel.

    Do not use a pipette to remove fluid as this may remove precipitated elastin.

    NB: *Ensure Precipitating Reagent is fully drained since excess liquid in the tube can interfere with elastin-dye binding.*

Formation of Elastin - Dye Complex

6. Add 1.00ml of Fastin Dye Reagent to all tubes, cap and mix contents using a vortex mixer.

7. Place the rack of tubes on a mechanical shaker (with gentle shaking) and incubate at room temperature for 90 minutes.

8. Centrifuge the tubes @ 13000 x *g* for 10 minutes and then drain the unbound dye into a waste container. *This isolates dye-labelled elastin as a pellet or coating on the inside tube surfaces.*

Recovery of Elastin-Dye Complex

9. Stand each tube with the tube opening facing down onto a clean, single thickness absorbent paper towel for **20 minutes**. *This allows residual dye to drain away from the elastin and gather around the tube opening.*

10. Gently take each tube and **keeping it in the inverted position at all times**, use a clean 'cotton swab, (or Q-tip) to remove any residual fluid droplets from around the opening of the tube.
    NB: *The elastin-dye complex can be observed as a reddish-brown deposit in the bottom and inside lower wall of the tube, (see website). Take care not to remove any elastin during fluid removal.*

Release, and Measurement of Elastin Bound Dye

11. Add 250µl of Dye Dissociation Reagent to each tube and place on a mechanical shaker (with gentle shaking) for approximately 10 min.

12. Vortex briefly, then transfer 150 µl of dye extract from each tube to a well in a 96-well, flat bottom microwell plate.

    Retain a map of the placement of samples, controls, and standards in the microplate.

13. Set the microplate reader to 513nm and measure the absorbance in 'endpoint' mode.

For graphing and data analysis proceed to Part C of this manual

PART C – Data analysis

Plotting the standard curve

1. Having collected the absorbance data, the value of any assay 'blank(s)' / negative controls should be subtracted to generate a set of 'blank-corrected' data. ***NB:*** *The use and subtraction of a suitable negative control is essential when using samples of cell culture media that contain added serum.*

2. The average values and any desired statistics for standards or samples can then be calculated, preferably via suitable software.

   Use these data to plot a graph of absorbance (*y-axis*) against elastin concentration (*x-axis*), as prepared from ***Table 1. 'Preparation of assay standards'***. A linear regression should be fitted to the curve since this matches the dye-elastin binding characteristics.

3. Consult your plate-reader or statistical software manual for instructions on how to calculate the concentration of unknown samples from a linear regression line.

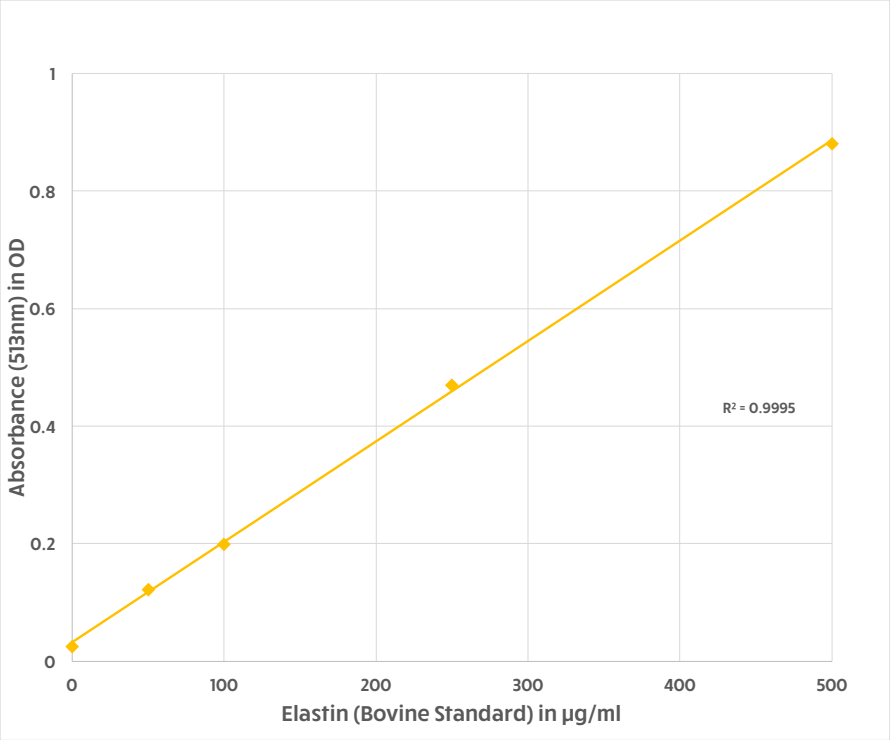
   The standard curve should exhibit the following:

   a. A linear plot, ideally with a R-squared value ≥0.99. An example is shown in **Figure 2.**, below.

   b. The '0' elastin standard should exhibit an absorbance (513nm) of between 0 – 0.1, depending on sample composition.

   c. Replicates should be close to ±5% of their main value.

Figure 2. Fastin Standard Curve (0 - 500µg/ml range)



Notes on multiple extractions / dilutions / larger sample volumes

a) If you have performed multiple extractions from one sample and assayed these individually, then they should be added together to calculate the **total** elastin content.

b) If samples were **diluted** prior to assay, then the calculated elastin content should be multiplied by the appropriate sample dilution factor.

c) If samples were **concentrated** (by using a larger sample volume), then the sample '*concentration factor*' should be first calculated by dividing the sample volume (*in µl*) by the volume of reference standard used (*100 µl*).

   Subsequently, the calculated elastin content of the sample should be then divided by the sample 'concentration factor'

   For example, if 200 µl volumes of sample were assayed, then the calculated elastin content (µl per ml) should be divided by a concentration factor of '2'. (200 µl sample volume /100 µl reference standard volume = a concentration factor of 2).

Troubleshooting - What if samples do not fall within the range of the standard curve?

Accurate quantification can only be achieved for samples that fall within the range of the standard curve:

- **Are your sample absorbances too HIGH?**

Samples with absorbance values greater than the upper range of the assay standards should be *diluted* and rerun.

- **Are your sample absorbances too LOW?**

If your samples have a calculated elastin content of ‹50µg/ml then you may wish to consider using a large sample volume in **step 1** of the General Protocol, followed by addition of an equivalent volume of Precipitating Reagent in **step 2** of the protocol. This results in the precipitation of a larger quantity of elastin for Fastin analysis. The change must be considered when calculating the elastin content of the samples.

Calculation of elastin content of solid tissue samples.

Many samples used with the Fastin assay take the form of 'oxalic acid' extracts, derived from solid tissue. The elastin content of these oxalic acid extracts can be directly calculated via the standard curve (*in µg/ml*). This is a primarily a measurement of the elastin concentration of the prepared liquid sample.

For comparison with other samples, it is useful to express the elastin content relative to the original tissue, in ***µg/mg of tissue*** (for *in-vivo* samples) or ***µg/growth area or volume*** (for *in-vitro* samples). This can be achieved as follows:

1) Calculation of elastin content of a tissue sample (*µg/mg)*

$$\frac{(C/1000)*V}{M}$$

C is the calculated concentration of elastin (calculated from the standard curve ***in µg/ml***).
V is the total volume of oxalic acid (***in µl***) added to the original sample (when extracting elastin).
M is the mass of original sample processed (***in mg***)

2) Calculation of elastin content per growth area (*µg/cm²)*

$$\frac{(C/1000)*V}{A}$$

C is the calculated concentration of elastin (calculated from the standard curve ***in µg/ml***).
V is the total volume of oxalic acid (***in µl)*** added to the original sample (for extraction of elastin).
A is the growth area that processed sample was derived from (***in cm²***)

Calculation of soluble elastin content in fluids

The concentration of soluble elastin in liquid samples (such as cell culture media) can be directly calculated (in *µg/ml*) using the linear regression curve.

PART D – Sample Preparation protocols

Example data can be found on the Fastin product webpage

General notes on Sample Preparation

Why do samples need preparation?

Fastin analysis requires liquid samples containing elastin in a soluble form.

For samples where elastin is already soluble (such as tropoelastin in samples of conditioned cell culture media), only minimal processing is required. However, samples of solid tissues or cellular ECM contain mature elastin which is hydrophobic and hence insoluble. Such samples require preparation to extract and convert the elastin to a soluble form which can be assayed.

This extraction is performed by heating the test samples in hot (95 – 100°C) oxalic acid for one hour.

Recommended extraction procedures for different types of samples are described in this section. For your convenience these have been separated into *in-vivo* and *in-vitro* protocols. *We suggest you use the flowchart on the Fastin assay QuickStart guide to select the correct protocol for your samples.*

- *Some protocols will require reagents or equipment not supplied with the kit. Customers should familiarise themselves with these requirements beforehand!*

- Modification or further optimisation may be required for some samples. If in any doubt, please feel free to contact us, we are always happy to advise!

Sample preparation guidelines

Following extraction/preparation, Fastin samples should be:

- Free of any particulate material, as this can interfere with colorimetric analysis.
- Prepared in oxalic acid (0.25M) or pH neutral tissue culture medium/buffer.

Additionally:

- Some samples may contain added surfactant, detergents, or 'non-standard' chemistries, for example – crosslinking reagents from 3D-scaffolds. For such samples we would recommend that a control sample of fresh (unused) buffer or sample is assayed, both with and without added assay standard. This will enable customers to confirm that these components do not interfere with the assay.
- Prepared samples may be stored frozen (-20°C to -80°C) prior to analysis. This conveniently allows samples to be harvested over different time periods and then assayed together. Frozen samples should be thawed slowly at 4°C and then vortexed to mix prior to assay.
- Both 'wet' (fresh or frozen) or 'Dry' (freeze-dried) tissues can be analysed and the elastin content expressed as ***µg elastin/mg wet tissue*** or ***µg elastin/mg dry tissue.***

I. Preparation of *in-vivo* samples

The following section provides guidance on how to prepare *in-vivo* derived samples for Fastin analysis. These are usually obtained by biopsy or dissection and may be processed as 'wet' tissue or freeze-dried for processing as 'dry' samples.

Such samples typically contain insoluble cross-linked elastin which requires extraction via heating (95 – 100°C) in the presence of oxalic acid. This generates *soluble,* cross-linked polypeptide elastin fragments of α-elastin that are suitable for assay.

Guidelines for *in-vivo* sample harvesting

Material sampled post-mortem should be collected as soon after death, in an aseptic a manner as possible.

1. Briefly wash the external surface of the tissue with sterile water or saline to remove any debris and blood.

   NB: *Lung and liver samples can be difficult to 'clean-up' as they are usually blood saturated. It is advisable to dice these samples and briefly rinse in cold phosphate buffered saline, before proceeding with preparation.*

2. If the sample contains attached adipose (fat) tissue, it should be trimmed off using a scalpel.

3. If elastin extraction is to be carried out at a later date, then samples should be weighed, placed into labelled, sealable containers and frozen (≤ 20°C) as quickly as possible. When defrosting, the frozen samples should be thawed slowly (in a refrigerator at 4°C) before subsequent processing.

4. Decide if elastin content of the test samples is to be expressed as 'dry weight' or 'wet weight'.

   If using dry weight then it will be necessary to take a representative sample, first obtain its wet weight and then dry the sample in an un-heated drying cabinet containing drying granules. Weigh samples daily until a constant dry weight value is obtained (most tissues are ~ 70% water).

5. For efficient elastin extraction the tissue samples should be 'diced' into small cubes, using a sharp scalpel. Alternatively, the tissue can be ground/homogenised (do not use any added buffer). Weigh prepared samples into a suitable tube or container.

   NB: *Non-homogeneous elastin distribution within the tissue can be a cause of sample variation. Provided there is sufficient tissue, this can be mitigated by selecting larger samples, or by pooling multiple smaller samples.*

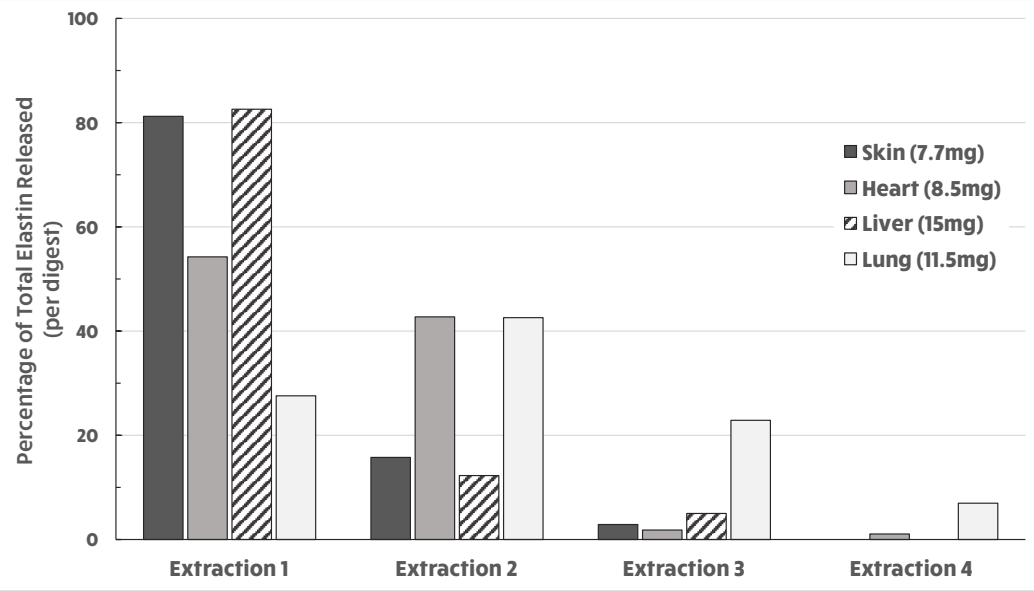
Optimising elastin extraction for different tissues

Some tissue material, such as that from foetal or immature animals, is readily solubilised after 1x or 2x oxalic acid extractions. However, tissue from mature or older animals, including aged human tissues, can require up to 3x extractions. An example of this approach using diced (non-homogenised) mouse tissue is shown in **Figure 3.**

When beginning to work with a new tissue type or source, we would suggest taking the time to identify the *optimal* number of extractions necessary to extract *all* elastin from the tissue. This can be achieved by performing 3x sequential extractions (on the same representative sample), then performing a trial Fastin analysis on these extracts.

If the 3rd extraction still contains significant elastin (such as the example of Lung tissue, in Figure.3, below), then we would suggest that the samples should be homogenised prior to extraction.

Figure 3. Sequential extraction of elastin from non-homogenised mouse tissues



Extraction of insoluble tissue elastin as water-soluble α-elastin

Tissue samples should be weighed before use. As a general guide, we suggest using between **10 - 20mg** of tissue and keeping a record of the weight. This will enable you to express the elastin content as ***µg elastin/mg wet tissue*** or ***µg elastin/mg dry tissue***.

1st Extraction

1. Weigh tissue samples of 10 – 20mg mass into labelled 1.5ml, heat-resistant screw-cap tubes (or other appropriate size), then add 0.25 M oxalic acid in the following proportions:

Oxalic Acid volume	Retain a record of the extract masses and volumes used to permit calculation of the tissue elastin content
75µl per mg of **WET tissue** *(can be fresh or frozen)*	
250µl per mg of **DRY tissue** *(such as freeze-dried tissue)*	

   NB: *A suitable volume of 0.25M oxalic acid extraction solution should be prepared by diluting the supplied 1.0M oxalic acid in the ratio 1:3. (i.e. To prepare a 10ml volume, add 2.5ml 1M oxalic acid stock to 7.5ml of diH2O).*

2. Add the screw cap and place tubes into a heating block or water bath with the thermostat set at 100°C for 60 minutes. *Vortex or invert tubes 2 - 3 times during heating to ensure efficient extraction.*

3. Following heating, remove the tubes from the heat and cool to room temperature.

4. Centrifuge tubes at 13000 *x g* for 10 minutes.

5. Use a pipette to carefully transfer the liquid supernatant to a fresh, labelled 1.5ml tube (take care not to disturb any residue at the base of the tube). This supernatant contains any solubilised sample elastin and is now ready for Fastin analysis. It can be assayed directly, or frozen (at ≤-20°C) for subsequent assay. *The residual material may contain further 'unextracted' elastin, we recommend performing further extractions to ensure that all elastin has been extracted.*

2nd / 3rd Extractions

6. To perform subsequent extractions, add the same volume of 0.25M oxalic acid (as added in step 1) to the residual tissue in the tube and repeat steps 2 - 5. Label extracts accordingly following step 5. *Multiple extractions should be assayed and the results included in calculations of tissue elastin content.*

II. Preparation of *in-vitro* samples

Elastogenesis is the process by which cells produce mature elastin, an integral part of the extracellular matrix. In this process, cells first export tropoelastin, a soluble form of elastin, together with fibrillin, which acts as a template for elastin deposition at the cell membrane surface. Over time, tropoelastin monomers aggregate into multimers and crosslink to become insoluble mature elastic fibres within the extracellular matrix.

The following sample preparation protocols enable Fastin analysis of soluble and insoluble forms of elastin produced *in-vitro* by cells within 2D (conventional) and 3D culture systems.

1) Elastin that has been secreted into the cell culture medium

Elastin can be secreted into cell culture media as the tropoelastin monomer. For assay purposes this can be considered 'soluble', requiring minimal preparation for assay.

2) Cell associated tropoelastin/elastin

In contrast, Cell surface & ECM-associated elastins should be considered 'insoluble' and will require solubilisation by oxalic treatment, prior to Fastin analysis.

Please see individual protocols for further detail.

Non-specific binding and cell culture components

If using cell culture scaffolds/constructs, be aware that some materials can release compounds that interfere with the Fastin analysis. If this interference is suspected, we suggest performing an additional "mock" extraction or incubation using a spare scaffold/construct and culture media only (without any added cells). This can be run as a control.

Analysis of Elastin secreted into culture media

During in-vitro cell culture, tropoelastin can be secreted into the culture medium. Customers wishing to measure elastin secretion into culture media should ensure they include suitable negative controls as this will maximise the sensitivity of the assay to low concentrations of elastin. *See the protocol below for further detail.*

Cells should be seeded in an appropriate vessel and grown under defined experimental conditions until ready, then:

1. Gently rock/swirl the cell culture plate or flask for 10 seconds to ensure the media sample is homogenous. Take care not to dislodge any adherent cells or spill media.

2. Remove the media sample using a pipette or needle and transfer to a suitable tube. Remove aseptically if cell culture is being maintained after sampling.

NB: *The general protocol specifies using 100µl samples, however since the concentration of secreted elastin is often close to the assay limit of detection it is possible to use larger volumes of culture media. This enables larger quantities of elastin to be precipitated and subsequently measured.*

If using larger sample volumes, they MUST be matched to a larger volume of Precipitating Reagent (see ***step 2.b*** *in the* ***General Protocol****). The larger sample volume must be accounted for during calculation of elastin content.*

3. Centrifuge the media sample(s) at 1500 x *g* for 10 mins to pellet any loose cells or insoluble material.

4. Transfer the clarified supernatant only (which will contain any soluble elastin), to a fresh, labelled tube.

   This is now ready for direct Fastin assay (see **step 1** of the **General Protocol**), alternatively the sample can be frozen (at ≤-20°C) for subsequent Fastin analysis.

Elastin analysis in serum-supplemented cell culture media samples

A 'matrix effect' may be observed (as high background absorbance values) when using media samples containing animal serum-supplements. We suggest that *where possible*, this should be minimised by using animal serum with a concentration in the medium of ‹2.5% v/V.

When using media samples that contain *any* added animal serum, it is recommended that customers implement a '*negative control*. This should comprise a sample of identical media that has NOT been cultured with cells. Following 'Assay Plate Measurement' (see General Protocol) the absorbance value for this negative control(s) should be subtracted from the sample absorbance values. This will remove the contribution of the matrix (serum) to any absorbance signal, enhancing sensitivity to the elastin component of the sample.

Analysis of cell-associated elastin

Cells should be seeded in an appropriate vessel and grown under defined experimental conditions.

Removal of Cells from Tissue Culture Flasks and from Microwell Plates

1. Aspirate or decant growth media, taking care not to dislodge attached cells.

2. Rinse the cells briefly with pre-warmed (37°C) PBS and then aspirate or decant to remove.

3. Add 1x Trypsin or alternatively a non-enzymatic treatment (*such as Sigma-Aldrich C5914 Cell Dissociation Solution*) to the monolayer, in sufficient volume to enable cell detachment. Incubate for 5-10 min at 37°C. *We suggest using a 1ml volume for a T-25 flask or 250µl/well for 12 well plates.*

4. Firmly tap the flask to release any attached cells from the growth surface into suspension. *If any cells are still adherent, maintain at 37°C for a further 5 minutes until detachment is confirmed.*

5. Transfer the cell suspension to labelled 1.5ml, heat-resistant screw-cap tubes (or other appropriate size), and centrifuge at 300 x *g* for 5 mins to pellet the cells.

6. Decant the supernatant, ensuring that the cell pellet is retained. Then add 400µl of 0.25M oxalic acid solution.

   NB: *A suitable volume of 0.25M oxalic acid extraction solution should be prepared by diluting the supplied 1.0M oxalic acid in the ratio 1:3. (i.e. To prepare a 10ml volume, add 2.5ml 1M oxalic acid stock to 7.5ml of diH2O).*

Conversion of Cell-associated Elastin to soluble α-Elastin

7. Place tubes into a heating block or water bath with the thermostat set at 100°C for 60 minutes. *Vortex or invert tubes 2 - 3 times during heating to ensure efficient extraction.*

8. Remove tubes and allow to cool before centrifuging at 13000 x *g* for 5 mins to pellet any insoluble material.

9. Transfer the now clarified supernatant (which contains any soluble elastin), to a fresh, labelled tube.

   This is now ready for direct Fastin assay (see **step 1** of the **General Protocol**), alternatively the sample can be frozen (at ≤-20°C) for subsequent Fastin analysis.