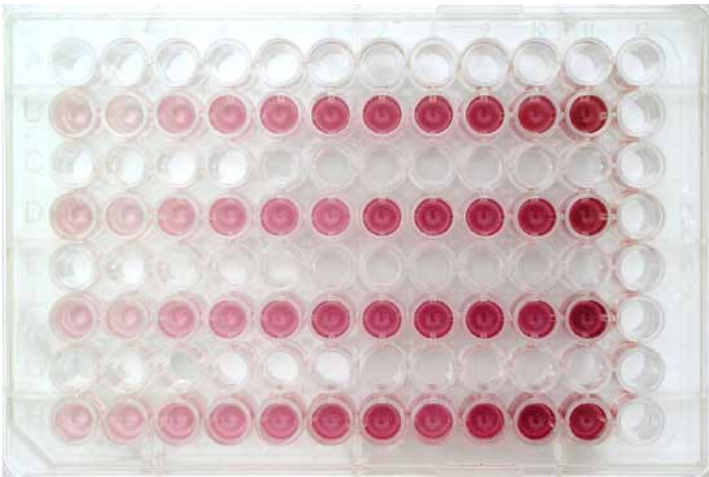


Fastin™

Elastin

Assay



**biocolor**

life science assays

[www.biocolor.co.uk](http://www.biocolor.co.uk)

[1] precipitated elastin (15 minutes),  
then centrifuged and supernatant  
removed



[2] elastin-dye complex after  
centrifuging and removing unbound  
dye



[3] recovery of elastin bound dye, after  
adding Dye Dissociation Reagent



# Fastin Elastin Assay

Time Req: 4 hours    Detection Limit: 5 $\mu$ g, (10 $\mu$ g/ml)

0  
mins



30  
mins



## Set up Assay:

Label a set of 1.5 ml microcentrifuge tubes. If sufficient test material is available run duplicate samples.

- [1] Prepare Reagent Blanks: 100 $\mu$ l of test solution solvent, (buffer / PBS / water / 0.25M oxalic acid).
- [2] a-elastin standard; (suggested, 12.5, 25.0 and 50.0  $\mu$ l duplicate aliquots).
- [3] Test samples; (*in vivo* derived tissue extracts or *in vitro* cell culture medium samples). Select aliquot volumes between 10  $\mu$ l and 500  $\mu$ l, (*to contain 5 to 70  $\mu$ g elastin*).
- [4] To each tube add an equal volume of Elastin Precipitating Reagent.
- [5] Cap tubes and briefly vortex to mix contents; leave for 10 minutes to complete precipitation of elastin.
- [6] Centrifuge tubes @ >10,000 x g for 10 minutes.
- [7] 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.

## Formation of Elastin - Dye Complex:

- [1] To all tubes add 1.00 ml of Dye Reagent.  
Cap tubes and initially mix contents by inverting the tubes.  
Next disperse the elastin precipitate using a vortex mixer.  
Then place the rack of tubes on a microplate shaker unit.  
Allow the reaction between the elastin and the dye to proceed for 90 minutes.
- [9] Centrifuge the tubes @ >10,000 x g for 10 minutes.

CONTINUED ON INSIDE BACK COVER OF MANUAL

**140  
mins**



**180  
mins**



**240  
mins**

### Recovery of elastin-dye complex:

- [10] Drain the tubes of unbound dye. While the tube is still inverted remove most of the remaining fluid from the tube by FIRMLY tapping the lip of the tube onto a single thickness of an absorbent paper towel. A 'cotton bud', (or Q-tip), can be useful in removing any fluid droplets from the rim of the tube. On returning the tube to the upright position, not more than 25µl of fluid should be found in the bottom of the tube.
- [11] The elastin-dye complex can be observed as a reddish-brown deposit in the bottom and inside lower wall of the tube.

### Release, and recovery, of the elastin bound dye:

- [12] To each tube add 250 µl of Dye Dissociation Reagent. Cap tubes and release the dye into solution with the aid of a vortex mixer. Repeat the vortex mixing after 10 minutes so as to ensure all bound dye has passed into solution.
- [13] Transfer the contents of each tube to a well in a ninety-six well flat bottom microwell plate. Ensure that a map has been prepared in the lab notebook to record which tube contents went into which well.

### Elastin measurement; (dye recovered):

- [14] Place microwell plate into the Microplate Reader. Select wavelength or colour filter nearest to 513 nm, (blue-green colour).  
Plot Reference Standards, (including Reagent Blanks), graph where both Absorbance and Elastin Concentration are known.  
Use this graph to determine or calculate the elastin content of the Test Samples.

### Notes:

- (a) Aim to achieve duplicates that do not exceed  $\pm 10\%$  of their mean absorbance value.
- (b) Comfort or coffee breaks can be obtained between Steps 7 & 8, during Step 8 and between Steps 11 & 12.
- (c) Like all dyes TPPS is photo-labile. Protect tubes/ microwell plate from direct sunlight or from strong overhead lighting.

**PLEASE READ THE MANUAL BEFORE USING THE ASSAY**

***Fastin*** <sup>™</sup>  
**ELASTIN**  
***Assay***

**TECHNICAL INFORMATION**

**Contents**

<b>Test material suitable for analysis</b>	<b>3</b>
Assay components and storage conditions required	3
Mode of action of the Fastin Assay	5
<b>Elastin Assay :</b>	<b>7</b>
Soluble elastin	8
Insoluble elastin	12
<b>Elastin History &amp; Information: Source References:</b>	<b>15</b>

**The *Fastin*™ Assay has been designed  
for *in vitro* research work only**

**Handle the  
Fastin Assay Kit  
using**

**GOOD LABORATORY PRACTICE**

**Read Manual Before Use**

***Fastin* Manual**

Not to be reproduced in part or in whole, without written permission,  
unless required for personal, non-commercial use.

**©Biocolor Ltd., 2007**

Fastin is a Trademark of Biocolor Ltd.

Published by

**Biocolor Ltd.**

**8 Meadowbank Road, Carrickfergus, BT38 8YF  
Northern Ireland.**

**[www.biocolor.co.uk](http://www.biocolor.co.uk)**

6th Edition 2007

# ***Fastin Assay***

## ***Manual***

### **Intended Applications:**

The Fastin Elastin Assay is a quantitative dye-binding method for the analysis of elastins extracted from biological materials.

The dye label employed is 5, 10, 15, 20-tetraphenyl-21, 23-porphine tetra-sulfonate (TPPS). For the structural form of the dye see Fig. 1.

### **Test sample material:**

Tissue extracts and cell culture medium.

Elastin forms that can be measured by the Fastin Assay:

- [i] soluble tropoelastins
- [ii] lathyrogenic elastins
- [iii] insoluble elastins, following solubilization to elastin polypeptides, [ $\alpha$ -elastin;  $\kappa$ -elastin]

The dye reagent binds to the 'basic' and 'non-polar' amino acid sequences found in mammalian elastins.

Due to the difficulty of obtaining sufficient quantities of tropoelastin the assay development was carried out using  $\alpha$ -elastin.

### **Test sample quantities:**

A sample volume of between 5 and 500  $\mu$ l is required, containing not less than 5  $\mu$ g and not more than 70  $\mu$ g elastin.

Samples with elastin of  $>70 \mu\text{g}/10 \mu\text{l}$  should be diluted with water, dilute buffer or PBS.

Samples with elastin of  $<5 \mu\text{g}/100 \mu\text{l}$  should be concentrated by freeze-drying or with the aid of an ultra filtration membrane.

### **Test sample composition:**

For analysis of soluble elastin, samples should be free of any particulate material (cell debris, insoluble extracellular matrix material). The presence of other soluble proteins or of complex carbohydrates does not interfere with the Fastin Assay.

Samples obtained by extracting elastin from tissues with oxalic acid (see page 12) are suitable for direct analysis: before use check that there is no insoluble material or turbidity present in the extracts

### **Fastin Assay Kit components:**

- [1] The **Fastin Dye Reagent** contains 5,10,15,20-tetraphenyl-21,23-porphine tetra-sulfonate (TPPS) in a citrate-phosphate buffer, that also contains ammonium sulfate and anti-microbials
- [2] **Elastin Precipitating Reagent** contains trichloroacetic acid and hydrochloric acid.
- [3] **Elastin standard** is a high molecular weight fraction of  $\alpha$ -elastin prepared from bovine neck ligament elastin. The  $\alpha$ -elastin standard is supplied as a sterile solution, concentration 1mg/ml, in 0.25M oxalic acid.
- [4] **Dye Dissociation Reagent** contains guanidine HCl and propan-1-ol

## **Recommended storage conditions for Assay Kit components:**

### **Unopened:**

All of the reagents have long term stability (at least 6 months), when stored at room temperature.

Do not freeze as complete re-solubilisation may not occur on thawing.

Avoid exposure of the Fastin Dye Reagent to direct sunlight.

### **Opened:**

**Reference Standard:** When stored at +4°C the  $\alpha$ -elastin standard is a clear transparent solution. On holding at room temperature the solution may be observed to become opalescent. This is due to the characteristic coacervation property of soluble elastin. On cooling, the process is reversible and the elastin solution again becomes transparent.

The full metal seal should not be removed from the vial; the contents can be sampled as follows:

- [1] Remove the centre metal disc only from the vial top.
- [2] Obtain aliquots from the vial by using a syringe fitted with a sterile hypodermic needle. The butyl rubber seal on the vial has a thin centre disc.
- [3] Do not return any unused aliquots to the vial.
- [4] The  $\alpha$ -elastin standard should be discarded if the solution becomes turbid.

### **Fastin Dye Reagent:**

The pH of this reagent is pH 7.5 and the dye label TPPS. To limit microbial growth, inhibitors have been added to the reagent. These agents, bromopol and sorbic acid, are compatible with the Fastin Assay; but are not 'universal' microbial inhibitors. Good laboratory practice and the storage of an opened bottle of Fastin Dye Reagent storage at 4°C can extend the shelf life of the reagent. DO NOT FREEZE.

### **Other components required, but not supplied:**

Capped 1.5 ml micro-centrifuge tubes and a set of variable volume micro-pipettors, with matching pipette tips.

For the microcentrifuge tubes a vortex mixer, to dislodge the protein pellet and then a mechanical shaker to provide gentle mixing of the elastin and the TPPS of the tubes.

A centrifuge, fitted with a 1.5 ml micro tube rotor head and capable of 10,000 x g.

A Microplate Reader, with a suitable colour filter (absorbance peak of TPPS occurs at 513 nm). See page 10 for further information on filter selection.

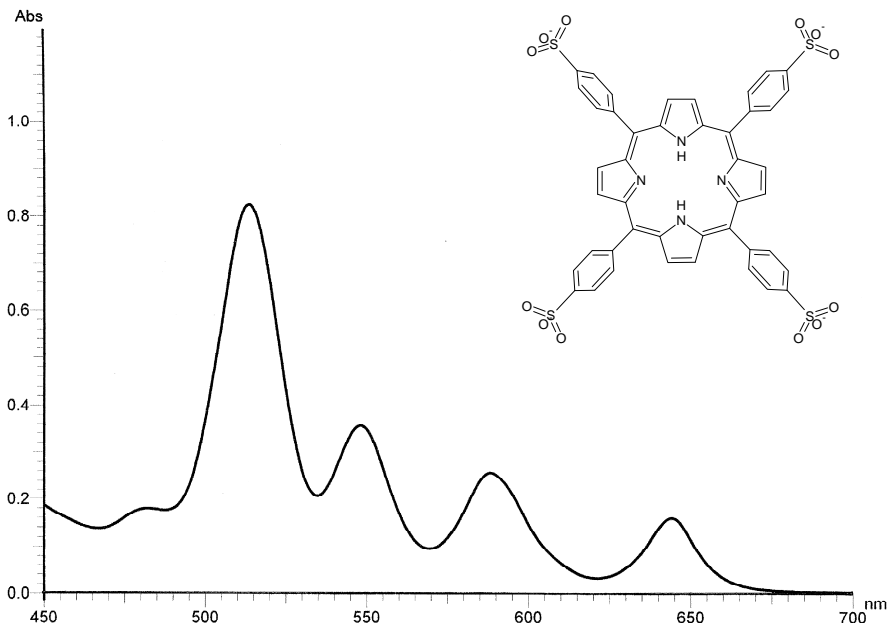
Magnetic Stirrer, with hotplate. A 500 ml glass beaker containing ~300 ml water and a spinning stir bar, (to prevent water bumping at 100°C), is convenient set-up for extracting elastin from tissue samples, (see page 12, section [b])

## Mode of action of the Fastin dye reagent with elastins:

The Fastin Dye Reagent contains a synthetic porphrin, 5,10,15,20-tetraphenyl-21,23-porphine, that is water soluble in the sulfonate form. The TPPS molecules contain four sulfate groups.

**Fig. 1**

*The visible absorbance spectrum and structural form of 5,10,15,20-tetraphenyl-21,23-porphine, tetra-sulfonate*



The affinity of TPPS for elastin was first observed when used as a 'vital stain' on live animals. Most tissues initially took up the dye, but with time only elastin retained the TPPS molecules. [Winkelman, J. (1962), *Cancer Res.* **22**, 589-596; Winkelman, J & Spicer, S. (1962), *Stain Technol.* **37**, 303-305].

The mode of action of TPPS with elastin remains uncertain. It may be due to shape-and-fit with the acidic dye being firmly retained by the basic amino acid side chain residues of elastin.

At pH 7.0 and 20°C, TPPS has been reported to occur as a dimer, producing an out-of-plane type conformational change [Schneider, W. (1975) *Struct. Bond.* **23**, 123].

# Elastin Assay

## MEASUREMENT OF SOLUBLE ELASTIN

---

### Set up assay:

To duplicate, labeled 1.5 ml microcentrifuge tubes add between 5 and 500  $\mu$ l of test samples, elastin standards and reagent blanks.

---

### Working standards:

It is recommended that with each Fastin Assay Kit, the **Elastin Standard** is initially run in duplicate at four concentrations; (0, 12.5, 25 and 50 $\mu$ l aliquots).

The standards along with the reagent blank can then be used to produce a straight line calibration curve from a selected Microplate Reader.

In subsequent assay batches, a minimum calibration requirement is duplicate 25  $\mu$ l aliquots of the elastin standard and the reagent blank. This secondary standard should give absorption values to within  $\pm 5\%$  of that defined by the standard curve.

When using a different measuring system, or following instrument servicing, a new standard curve should be prepared.

---

### Test samples:

With test samples where the approximate elastin concentrations are as yet unknown, initially try single 50 $\mu$ l aliquots for the first trial .

If the absorbance readings are found to be  $>1.0$ , (after subtraction of the reagent blank value), repeat assay using a smaller test sample aliquot.

If in initial trial aliquots produced absorbance values of  $<0.05$ , (after subtraction of the reagent blank value), the test sample contains less than 5  $\mu$ g elastin and will require a larger sample aliquot or concentration before being re-assayed.

For reliable and accurate results all test samples should have their absorbance readings within the range of the Elastin Standards that were plotted on the calibration curve.

---

## START ASSAY:

---

### Elastin isolation:

The **Precipitating Reagent** has been developed to perform this elastin recovery; the reagent should not be diluted with sample volumes greater than in a ratio of 1:1

The reagent can be pre-cooled to  $<5^{\circ}\text{C}$  (it is convenient to store this reagent in the refrigerator so that it is ready for use).

- [1] To tubes, containing Standards or Test Samples add an **equal volume** of the Elastin Precipitating Reagent.
- [2] Tubes are capped and the contents mixed, by inversion and then held for about 10 minutes.

### Recovery of elastin:

- [3] Following the precipitation of the elastin, the microcentrifuge tubes are centrifuged for  $>10,000 \times g$  for 10 minutes, to pack the precipitated elastin.
- [4] Remove tubes from the centrifuge, uncap and carefully invert, to drain the liquid contents into a waste beaker. While inverted remove any remaining fluid from the top of the tubes by tapping the tube onto an absorbent paper towel.  
  
A 'cotton bud', (or Q-tip), can be useful in removing any fluid droplets from the rim of the tube.

On returning the tube to the upright position, not more than  $25\mu\text{l}$  of fluid should be found in the bottom of the tube.

*Low concentrations of elastin can be difficult to 'see' as it occurs as a translucent gel.*

*Photographs on the outside back cover of this manual:*

*The top photograph required  $250 \mu\text{g}$  of  $\alpha$ -elastin to visually display the protein pellet.*

*The middle and bottom photographs were obtained using  $50 \mu\text{g}$   $\alpha$ -elastin samples.*

## Reaction of the elastin with the Fastin dye:

---

[5] Add 1.0ml of the **Fastin Dye Reagent** to each tube.

[6] Cap the tubes and use a vortex mixer to bring the elastin gel precipitate into solution with the Dye Reagent.

During centrifugation ( $>10,000 \times g$ ) the precipitate will have been compacted and may require 2 or 3 short periods with the vortex mixer to bring the elastin into solution.

Lower  $g$  forces ( $<7,500 \times g$ ) produce less well packed precipitates, but carries the risk of partial loss of some of the precipitate during the removal of the un-bound dye supernatant.

[7] The elastin and the Dye Reagent are allowed to interact for 90 minutes.

Gentle mechanical mixing is recommended during this period to ensure maximum elastin dye binding.

Any form of mixer, to which the microcentrifuge tubes or rack can be attached is suitable.

If no suitable mixer is available, then mix the tube contents at  $\sim 10$  min intervals by manual inversion or using a vortex mixer.

For optimum results the above conditions should be standardized.

Time periods of less than 90 minutes for reacting elastin with TPPS are not recommended.

---

## Recovery of the elastin-dye complex:

Following the dye binding step the elastin-dye complex formed becomes insoluble in the presence of ammonium sulfate within the Fastin Dye Reagent.

[8] The elastin-dye complex is separated from the remaining soluble unbound dye by centrifuging the tubes ( $>10,000 \times g$  for 10 minutes).

[9] The tubes are uncapped and the supernatants discarded. Any remaining fluid is removed by firm tapping of the inverted tubes onto a paper towel.

While inverted remove any remaining fluid from the top of the tubes by tapping the tube onto an absorbent paper towel. While the tube is still inverted remove most of the remaining fluid from the tube by FIRMLY tapping the lip of the tube onto a single thickness of an absorbent paper towel. A 'cotton bud', (or Q-tip), can be useful in removing any fluid droplets from the rim of the tube.

Visual inspection should reveal a red residue within the elastin standard tubes and, hopefully also in the test sample tubes.

---

### **Release of the elastin bound dye:**

[10] To each tube add 250  $\mu$ l of **Dye Dissociation Reagent**.

Cap the tubes and bring the elastin-bound dye into solution using a vortex mixer.

Two brief mixing periods are usually more effective than one long mixing period.

Tubes should not be uncapped until transfer to the wells of a 96-well microplate for absorbance measurement.

The dye extract is stable for several hours, but if readings are to be delayed store the tube rack containing the microcentrifuge tubes in a light-proof container or cupboard.

---

### **Elastin measurement:**

The elastin content of the assayed samples is determined by the amount of bound dye released from the elastin.

Transfer the complete 250  $\mu$ l contents of the labeled micro-centrifuge tubes to wells of a 96 well microplate, (with flat-bottom wells to reduce light scatter effects).

The absorbance peak of TPPS in the Dye Dissociation Reagent occurs at 513 nm.

Although the instrument can be set to zero using the reagent blank, it is usually better to determine the absorbance of the blank as a quality control check of the assay.

Duplicates (blanks, standards and test samples) should not exceed  $\pm 10\%$  of duplicate means.

With practice the analyst can usually obtain this after processing one or two batches of assays.

When any set of duplicate test sample readings exceed  $\pm 10\%$  of their mean value the sample should be checked for clarity and re-assayed.

Check the colour filter options that are available for the Microplate Reader; a blue green filter will probably be found to be suitable.

Initially also try the filters on either side of the selected filter; read the blank and standards with each of the three filters.

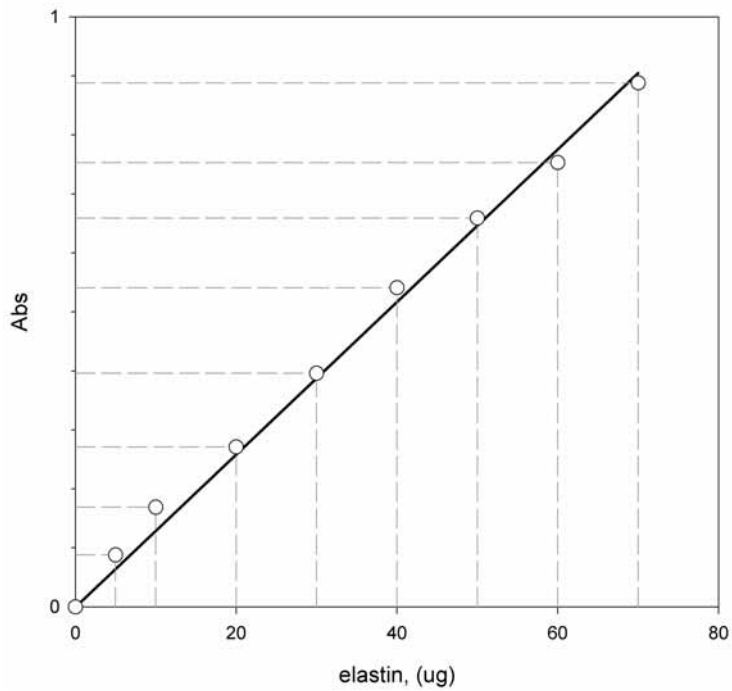
Plot standard curves for each of the filters and select the optimum curve based on that which gave the highest absorbance readings of the standards, *and produced* a straight line fit for the standard readings.

For further information on colour filter selection see Fig. 3 (page 14)

---

### **Elastin concentration in Test Samples:**

- [1] The duplicate absorbance readings of the reagent blank and the three, or more, sets of elastin standards are used to produce a standard curve.
  - [2] Subtract the reagent blank mean absorbance value from the absorbance means of each duplicate set of elastin standards and test samples.
  - [3] Plot the elastin standard values; vertical axis 'Absorbance' and horizontal axis; 'elastin concentration; ( $\mu\text{g}$ )'.
  - [4] Obtain the best fit line through these plotted points, [Fig. 2].  
The line should pass through zero (0.0 absorbance, 0  $\mu\text{g}$  elastin).  
Do not extend the line beyond the highest plotted point.  
The plot points should be within  $\pm 5\%$  (absorbance) of the fitted line.
  - [5] The concentration of elastin present in the test samples can now be determined.  
Take the mean of each duplicate test sample after subtracting the reagent blank value.  
The equivalent elastin concentration for the absorbance value obtained is then read from the standard curve.
  - [6] The quantity of elastin found in the aliquot taken for analysis can then be multiplied to give the elastin concentration present, either in 100  $\mu\text{l}$  sample or 1ml test sample.
  - [7] As the total extract volume has been logged, (of the pooled extracts), from a known weight of tissue, (wet or dry milligrams). It is possible to express the elastin concentration as; ' $\mu\text{g}$  elastin per mg tissue'
-



***Fig. 2.***

*Elastin Standard Curve;*

Microplate Reader 96 well format, 250  $\mu$ l/well.

For further information see Fig. 3.

## MEASUREMENT OF INSOLUBLE ELASTIN

The Fastin Assay can be used to measure insoluble cross-linked elastin. Insoluble elastin is extracted from tissue in the form of soluble cross-linked polypeptide elastin fragments.

Insoluble elastin can be converted into either soluble  $\alpha$ -elastin or  $\kappa$ -elastin. After extraction the samples can be assayed by the procedure described for soluble elastin.

### Conversion of insoluble elastin to water soluble $\alpha$ -elastin

- [a] Tissue samples are weighed (decide if the elastin content is to be expressed as wet weight or dry weight, ( $\mu\text{g}/\text{mg}$ ), of tissue).
- Cut tissue into small fragments for extraction.
- Place the weighed samples into centrifuge tubes ( $\sim 10 \times 2 \text{ cm}$ ) and add  $\sim 20$  volumes of 0.25 M oxalic acid (assuming tissue density is  $1.00 \text{ gm}/\text{cm}^3$ ).
- [b] The tubes are then placed into a boiling water-bath (or a metal heating block with the thermostat set at  $100^\circ\text{C}$ ), for 60 minutes. Do not tighten tube caps.
- [c] Remove the tubes from the heat and cool to room temperature.
- Centrifuge at  $\sim 3000 \text{ rpm}$  for 10 minutes.
- Pipette off the liquid and retain this extract in labeled containers.
- [d] To the residual tissue in the tubes add a further 20 volumes of 0.25 M oxalic acid and again heat for 60 min. Up to three heat extractions should be initially used to check that complete solubilisation of the tissue elastin has occurred
- [e] Some tissue material, such as that from foetal or immature animals, can be solubilised after one or two extractions. Tissue from mature or old animals, including aged human tissues, may require up to three extractions.
- [f] Initially when using new test material retain each of the oxalic acid extracts separately and analyze each for elastin; to establish that elastin extraction was quantitative. The last extract should contain no elastin.
- Elastin extracted from mature tissue, (elderly adult human tissue), will often produce yellow coloured extracts.
- [g] The tissue elastin in the form of  $\alpha$ -elastin which has an average molecular weight of 60-84 KDa. The extract can now be directly assayed using the procedure described for soluble elastins.
- Retain a record of the extract volumes to permit calculation of the tissue elastin content.

### **Conversion of insoluble elastin to soluble $\kappa$ -elastin:**

In this method, insoluble elastin is solubilised using ethanolic potassium hydroxide, (1 M KOH in 80% ethanol)

The elastin solubilisation reaction is carried out at 37°C for two hours only. The excess alkali in the pooled extracts is then removed by dialysis against water.

The solubilised elastin recovered has been termed Kappa-elastin ( $\kappa$ -elastin). This high molecular weight fraction, (< 50KDa), is composed of elastin polypeptides that have 'similar' properties to that of  $\alpha$ -elastin.

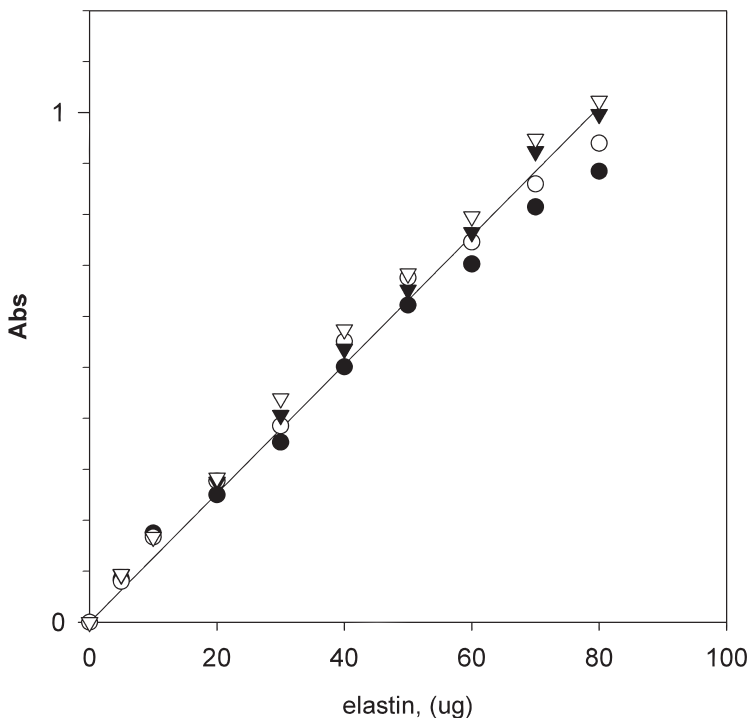
Further details on how to prepare  $\kappa$ -elastin can be found in the following reference Moczar, M., Moczar, E. & Robert, L. (1980), *Frontiers Matrix Biol.* **8**, 174.

### **Tropoelastin, the native monomer form of elastin:**

Tropoelastin, as exported from mammalian cells, has a molecular weight between 62 – 72 KDa.

This is the elastin form that maybe found in cell culture medium during *in-vitro* cell culture.

Tropoelastin avidly binds to a co-exported microfibril glycoprotein to form 'elastic matrix'. This associated glycoprotein is therefore a common component of many tropoelastin preparations.



**Fig. 3.**

*Microplate Reader colour filter selection*

The graph displays the results obtained, using two batches of Elastin Standard. These reference solution were used to produce the 'standard curves' plotted in the figure. Each sets of 'standards' were run in duplicate and the 'Absorbance' measured using two different Microplate Readers;

[1] Awareness Technology Inc., model Multi-well Reader II: Filter selected; 545 nm

[2] Bio-Tek Instruments Inc., Model EL 311: Filter selected; 550 nm.

The [1] data has been plotted using white symbols.

For [2] data was plotted using black symbols.

The 'Reagent blank' mean absorbance value was 0.385 for [1] and 0.162 for [2], when these values were subtracted from the 'standard readings' the blank corrected absorbance values of the elastin standards were similar for both Microplate Readers.

The photograph of the front cover of the manual is a visual image of the microwell plate that was used to produce this graph.

## ELASTIN SOURCE REFERENCES

### Biochemistry, biophysics; preparation, extraction & analysis

**Cunningham, L.W. & Frederiksen, D.W.** [1982], Eds of '*Elastin Structure and Biosynthesis*'. Methods of Enzymology, **82**, 559-743.

**Kreis, T & Vale, R.** [1993] Eds of '*Guidebook to the Extracellular Matrix and Adhesion Proteins*'. Oxford University Press, Oxford.

**Mecham, R.P.** [1986] Ed of '*Regulation of Matrix Accumulation*'. Academic Press, New York.

**Robert, L. & Hornebeck, W.** [1989], Eds of '*Elastin and Elastases*' in two volumes. CRC Press Inc. Boca Raton, Florida.

**Royce, P.M. & Steinmann, B.U.** [1993] Eds of '*Connective tissue and its heritable disorders; molecular, genetic and medical aspects*'. Wiley-Liss, New York.

**Sandberg, L.B., Gray, W.R. & Franzblau, C.** [1977] Eds of '*Elastin and Elastic Tissue*', (Advances Exper. Med. Biol. Series, Vol 79), Plenum Press, New York.

**Uitto, J., & Perejda, A. J.** [1987], Eds of '*Connective Tissue Disease. Molecular Pathology of the Extracellular Matrix*'. Marcel Dekker, New York.

## Other Assays available from Biocolor

### APOPercentage **Apoptosis** Assay

For mammalian cells that have a conventional phospholipid composition membrane. Assay is not recommended for non-mammalian cells and is not suitable for neural cells. Requires an inverted microscope, (magn. x 100), for detection and a microplate reader for measurement. Digital microphotograph analysis option using Adobe Photoshop.

*Assay sensitivity; single apoptotic cell      Assay run time; 1 hour*

### Sircol **Soluble Collagen** Assay

Suitable for monitoring collagen production during *in-vitro* cell culture or of recent *in-vivo* synthesis from tissue samples; such as wound healing and skin grafts research. Fibrillar collagens, [type I, II, III, V & XI] and basement membrane collagen, [type IV]. assay for acid-soluble, (cold 0.5 M acetic acid), mammalian collagens.

*Assay sensitivity; 2.5ug      Assay run time; 1 hour*

### Blyscan **Sulfated Glycosaminoglycans** Assay

For analysis of sulfated glycosaminoglycan components of proteoglycans including decorin, biglycan, fibromodulin, aggrecan, syndecan, betaglycan. **Galactosaminoglycans:** chondroitin sulfates, dermatan sulfate. **Glucosaminoglycans:** heparan sulfate, heparin, keratan sulfate. Test material; cell culture medium, amniotic fluid, urine, synovial fluid and tissue extracts.

*Assay sensitivity; 0.5ug      Assay run time; 1 hour*

FOR FURTHER INFORMATION AND UPDATES VISIT OUR WEBSITE

[www.biocolor.co.uk](http://www.biocolor.co.uk)