bio col or life science assays

# Fastin™ Quickstart Guide

Fastin is a colorimetric, quantitative dye-binding assay for the analysis of elastin extracted from *in-vivo* & *in-vitro* sources.

## First Steps

**Plan:** Use the **flowchart** on the back of this guide to select a suitable sample preparation protocol. The selected protocol should be read in combination with the indicated sections of the product manual.

**Prepare:** Ensure you have access to the necessary supporting equipment, indicated on **p6** of the product manual, and in the sample preparation protocol. Samples should be harvested and prepared as indicated.

**Proceed with the General Protocol...**

A

**General Protocol**
(see page **8-10** of manual for further detail)

## 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 12). 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.

**Table.1 Preparation of Reference 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.

B

## Precipitate elastin

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 **Precipitating Reagent** to all tubes containing such controls / samples.

**OR**

**b. Culture medium samples:**
Add 1.5x the volume (150 µl) of **Precipitating Reagent** to tubes that contain cell culture medium.

3. Cap the tubes and briefly vortex to mix contents; then place in the fridge at 4°C for 15 minutes.
4. Centrifuge tubes @ 13000 x g for 10 minutes. This causes precipitated elastin to form a pellet or coating on the inside tube surfaces.
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 an absorbent paper towel.

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

C

## Dye-labelling of Elastin

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.

**Recovery of Elastin-Dye Complex**

9. Stand each tube with the tube opening facing down onto a clean absorbent paper towel for **20 minutes**.
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. Take care not to remove any elastin during fluid removal.*

D

## Release & 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 the product manual.**

# Fastin Sample preparation & analysis flowchart

This flowchart can be used to identify the most appropriate protocol to prepare samples for Fastin analysis. *Full details can be found in* ***Part D*** *of the product manual.*

## What is the source of sample material?

See **p16** of manual for general information on samples. Species applicability information can be found on **p4** of the manual.

### *in-vivo sources?*

Such as: Skin, tendon, blood vessels, heart, etc

*see* ***p17*** *of manual for information on dissection, preparation & storage*

**Select tissue samples (10-20mgs) & transfer to heat resistant, screw-cap tubes**

***If using WET tissue, add***

*75µl 0.25M oxalic acid per mg of* ***tissue***

*See* ***p19*** *of manual*

***If using DRY tissue, add***

*250µl 0.25M oxalic acid per mg of* ***tissue***

*See* ***p19*** *of manual*

**Convert tissue Elastin to soluble α-Elastin**

using Oxalic Acid (100°C for 60 mins)

*Follow protocol on* ***p19*** *of manual*

**Repeat extraction until all elastin is extracted in soluble form**

*See* ***p19*** *of manual*

**OR**

### *in-vitro sources?*

Such as 2D or 3D cell culture

*see* ***p20*** *of manual*

**Are you measuring soluble tropoelastin in cell culture media?**

Prepare media for analysis

*Follow protocol on* ***p21*** *of manual*

**OR**

**Are you measuring cell associated elastin ?**

Remove cells from growth surfaces using Trypsin or a non-enzymatic treatment

*See* ***p22*** *of manual*

Convert Cell-associated Elastin to soluble α-Elastin

*Follow protocol on* ***p22*** *of manual*

## Perform ASSAY

Follow General Protocol on **p8** of manual

## *Analyse Data*

*see* ***part C*** *of manual*

**Are your sample absorbances too low to fit on the standard curve?**

(‹50µg/ml)

Consider using a larger extracted sample volume

*see* ***p13*** *of manual*

**Troubleshooting**

**Results!**

**Are your sample absorbances too high to fit on the standard curve?**

(›500µg/ml)

Dilute samples before assay

*see* ***p13*** *of manual*

**Troubleshooting**

Download a fresh copy @

**www.biocolor.co.uk/ecm-assays/fastin-elastin-assay**