

SARCOMERE LENGTH

PREPARATION OF MUSCLE FOR LASER DIFFRACTION:

1. Excise, in triplicate, small pieces (3.0 x 3.0 x 2.0 cm) of muscle with the fibers running longitudinally. Place the cores in scintillation vials. Add 5% glutaraldehyde solution and fix for 4 hours at 4°C.
2. Pour off the glutaraldehyde solution and replace with the 0.2 M sucrose solution. Fix overnight at 4°C. Cores can be stored at 4°C for up to 7 days in this solution.

SARCOMERE LENGTH DETERMINATION:

Sarcomere length is determined by the following equation (Cross et al., 1981)

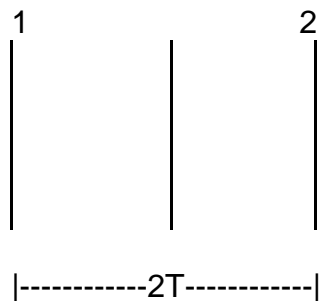
$$\mu = \frac{0.6328 \times D \times \sqrt{\frac{T^2}{D} + 1}}{T}$$

D = distance from specimen to diffraction pattern screen in mm
(preferably 100 mm)

T = spacing between diffraction bands in mm**

0.6328 = 632.8 (the wavelength of the laser) x 10⁻³

**A diffraction pattern will be projected on the screen as follows:



The distance between bands 1 and 2 is equal to 2T in the formula.

T = 1/2 the distance from 1 to 2.

Measure from 1 to 2 in mm and then divide by 2.

SOLUTIONS:

(Koolmees et al., 1986)

0.1 M NaHPO₄ buffer at pH 7.2

	<u>1 liter</u>	<u>2 liters</u>
Na ₂ HPO ₄ (mw 141.96)	10.18 g	20.36 g
NaH ₂ PO ₄ (mw 137.99)	3.91 g	7.82 g

Dissolve in distilled water, pH and bring up to volume. Store at 4°C.

5% Glutaraldehyde in 0.1 M NaHPO₄ buffer at pH 7.2

Glutaraldehyde (25%) 200 mls/liter

Bring up to 1 liter with 0.1 M NaHPO₄ buffer. Store at 4°C.

0.2 M Sucrose in 0.1 M NaHPO₄ buffer at pH 7.2

	<u>1 liter</u>	<u>2 liters</u>
Sucrose	68.46 g	136.92 g

Dissolve and bring up to volume with 0.1 M NaHPO₄ buffer.
Store at 4°C.

References

Cross, H. R., R. L. West, and T. R. Dutson. 1981. Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. *Meat Sci.* 5:261-266.

Koolmees, P. A., F. Korteknie, and F.J.M. Smulders. 1986. Accuracy and utility of sarcomere length assessment by laser diffraction. *Food Microstr.* 5:71-76.