Texas Tech University

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 four hours at 4°C and must be done in exhaust hood.

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 seven days in this solution, this must be done in an exhaust hood.

3. Remove sample from vial and excise several fibers using tweezers.

4. Place fibers on a clean microscope slide.

5. Spread fibers apart and add a small amount of sucrose solution to the slide (~ 10μm).

6. Once solution is added place cover slip on slide. **Once a slide is fixed it must be measured within an hour.**

7. Place slide on laser platform and move around until you see the laser diffract on the sarcomere (see below).

8. Measure distance between sarcomeres (mm) and record.

9. Move slide to another diffraction point and repeat. (Measure a total of six sarcomeres).

Sarcomere Length Determination

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

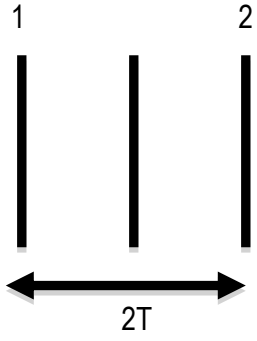
   $\mu = (.6328 \times D \times (\sqrt{(T/D)^2} + 1))/T$

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

   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 = ½ the distance from 1 to 2.
Measure from 1 to 2 in mm and then divide by 2.
Measure a total of 6 sarcomeres.

Solutions
(Koolmees et al., 1986)

1. 0.1 M $NaHP_4$ buffer at pH 7.2

	1 Liter	2 Liters
Na_2HPO_4 (mw 141.96)	10.18 g	20.36 g
NaH_2PO_4 (mw 137.99)	3.91 g	7.82 g

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

2. 5% Glutaraldehyde in 0.1 M $NaHPO_4$ buffer at pH 7.2.

   Glutaraldehyde (25%) 200 mls/liter

   Bring up to 1 liter with 0.1 M $NaHPO_4$ buffer. Store at 4°C.

3. 0.2 M Sucrose in 0.1 M $NaHPO_4$ buffer at pH 7.2

	1 Liter	2 Liters
Sucrose	68.46 g	136.92 g

Dissolve and bring up to volume with 0.1 M $NaHPO_4$ buffer.
Store at 4°C.

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

Koolmeers, 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.