

Papillary muscle isolation protocol

1. Prepare Arrest and Diffusion solution, filter sterilize for solutions made in advance and for any experiment to last longer than 6 hours.

Arrest solution

Chemical	Concentration (mM)	Per 1000ml (g)	Per 500 ml (g)
NaCl	137.2	8.02	4.01
KCl	15.0	1.118	0.559
MgCl ₂	1.2	0.244	0.122
Na Acetate	2.8	0.2296	0.115
Taurine	10.0	1.252	0.626
CaCl₂	1.0	0.147	0.074
Glucose	10.0	1.804	0.902
Hepes	10.0	2.383	1.192
BDM (optional)	20.0	2.022	1.011

Diffusion Solution

Chemical	Concentration (mM)	Per 1000ml (g)	Per 500 ml (g)
NaCl	137.2	8.02	4.01
KCl	5.0	0.373	0.186
MgCl ₂	1.2	0.244	0.122
Na Acetate	2.8	0.2296	0.115
Taurine	10.0	1.252	0.626
CaCl₂	2.0	0.294	0.147
Glucose	10.0	1.804	0.902
Hepes	10.0	2.383	1.192

2. Set out surgical instruments and prepare dissection apparatus and bath with arrest solution. Prime tissue chamber and diffusion lines with diffusion solution up to the stop cock. Fill the chamber with arrest solution.
3. Obtain and weigh mouse. Anesthetize mouse with Isoflurane and euthanize quickly by cervical dislocation. Open chest, isolate heart and inject left ventricle with arrest solution.
4. Remove heart with large portion of aorta intact, place in dissection bath.
5. Cannulate aorta and perfuse heart with arrest solution until coronaries are clear of blood. Pin heart into position.
6. Carefully open right ventricle and remove tissue flap
7. Identify a long, thin, unbranched papillary muscle. (Optional, useful for passive mechanical experiments: Remove fluid from around the muscle and spray with titanium dioxide surface markers). Dissect the muscle from the ventricle by cutting a small section of the valve to which the muscle is attached, and then free the muscle by cutting out a chunk of the septal wall attachment at the base of the muscle. Pierce a small hole in the valve with a needle to facilitate mounting in the tissue culture chamber.
8. Carefully transfer the free muscle to the chamber and position between the basket and titanium hook and align the muscle for isometric twitch measurements.
9. Run oxygen in the chamber or oxygenate the diffusion solution.
10. Flush the arrest solution from the chamber and replace with diffusion solution.
11. Take a preliminary length measurement of the unloaded muscle using a micro ruler. Record initial length
12. Stretch muscle to the minimum length at which a small force can be measured. Measure this muscle length and record. Use a field potential between 20 and 30 volts, about 15% above the minimum voltage at which the muscle is contracting reliably. Stimulate at 0.2 Hz using 5ms square pulses for at least 1 hour to equilibrate.

13. Completely unload the muscle. Take another length measurement, compare to initial measurement, and Record as L_0 .
14. Find L_{max} (the length at which the maximum force is developed)
 - a. Stimulate the muscle at the reference frequency, 1 Hz, stretch the muscle watching as the force increases until there is no change in force generation.
 - b. Decrease the length and repeat procedure two more times to precondition, then record (F4) the third trace.
 - c. From the force trace, determine L_{max} and record this value.
15. Begin Stress-Frequency Relationship (SFR) experiment:
 - a. Return the muscle to L_{max} . Field stimulate at 0.1 Hz. Allow actively developed stress several minutes to stabilize then record the force for eight twitch cycles.
 - b. Repeat for each of the following frequencies: 0.5, 1.0, 2.0, 3.0, and 4.0 Hz
16. Determine post-rest potentiation:
 - a. Reduce the stimulation frequency from the reference, 1 Hz, to 0.33 and measure the immediate force increase.
 - b. Repeat for 0.20 and 0.00 Hz. The rest interval will thus be varied between 3 and 10sec.
17. Measure the width, length, and thickness of the muscle with a micro ruler. Take video images of the muscle and titanium hook (with known length and thickness for reference) for measurement if preferred.
18. Loosen the muscle from the chamber attachments and clean up.
19. Save data and remove files from computer.
20. Label video tape and set aside for data analysis. Use the length measurements saved on the video tape to normalize force data and find the change in length ($L-L_0$).

DATA ANALYSIS

1. Record L_0 from the experimental notes. Or, define L_0 as the distance between two surface markers on the unloaded muscle measured from the video image.
2. Plot the passive force: $L-L_0$ (ΔL). Note that the passive force should be plotted from the raw data as Passive Force – Offset
3. Plot the active force: Active force= Total force – passive force. Plot against ΔL .
4. Normalize above passive force data to plot Stress v Strain.
5. Plot active force against Frequency
6. Plot active force against Length