

Standard Operating Procedure for Contractile Properties Testing *in vitro*

Equipment and chemicals

- All chemicals listed below were purchased from Sigma Chemical Company.

NaCl

KCl

MgSO₄

NaH₂PO₄

CaCl₂

NaHCO₃

α -D-Glucose

d-tubocurarine chloride

Preparation of Concentrated (10x) Krebs-Ringers stock solution

10x Salt Stock (in 1000 ml deionized water)

68.96 g NaCl

3.5 g KCl

1.4 g MgSO₄

1.5 g NaH₂PO₄

10x Calcium Stock (in 1000 ml deionized water): 2.78 g CaCl₂

10x Bicarbonate Stock (in 1000 ml deionized water): 21.0 g NaHCO₃

*Stock solutions stored in the refrigerator

Final Concentrations of Krebs-Ringers Working Solution

118 mM NaCl

4.7 mM KCl

1.2 mM MgSO₄

1.3 mM NaH₂PO₄

2.5 mM CaCl₂

25 mM NaHCO₃

10 mM α -D-Glucose

Preparation of Krebs-Ringers working solution

Working solution is prepared daily and discarded at the end of the experiment. A working KRS is prepared by adding 100 ml of 10x salt stock + 200 ml deionized water. Then, add 100 ml of 10x calcium stock + 200 ml deionized water. Finally, add 100 ml of bicarbonate stock. Add 1.8 grams of α -D-glucose and 0.017 grams d-tubocurarine chloride and fill with deionized water to \leq 1000 ml total volume.

- Oxygenate Ringer's solution for 10 minutes with 5% CO₂ and 95% O₂.
- Adjust pH to 7.4 using 1N HCL or 1N NaOH.
- Fill to 1000 ml total volume