

## **Slicing Buffer** for transport and slicing

according to Brandenburger et al. (Cardiovasc. Res., 2012), Fischer et al. (Nat. Commun., 2019)

Substance	Amount (g) for			Concentration (mM)
	1 L	2 L	5 L	
NaCl	8	16	40	136
KCl	0.4	0.8	2	5.4
MgCl <sub>2</sub> *6H <sub>2</sub> O	0.2	0.4	1	1
NaH <sub>2</sub> PO <sub>4</sub> *H <sub>2</sub> O	0.046	0.092	0.23	0.33
Glucose*H <sub>2</sub> O	2	4	10	10
CaCl <sub>2</sub> *2H <sub>2</sub> O	0.13	0.26	0.65	0.9
BDM	3	6	15	30
HEPES	1.2	2.4	6	5

Adjust pH to 7,4 using 1M NaOH. Sterilize by filtration. Can be kept at 4°C for months.

### Materials:

NaCl: Sigma, S5886-1kg, 1kg  
 KCl: Merck, 1.04933.0500, 500g  
 MgCl<sub>2</sub>\*6H<sub>2</sub>O: Applichem, A1036,0500, 500g  
 NaH<sub>2</sub>PO<sub>4</sub>\*H<sub>2</sub>O: Merck, 1.06346.0500, 500g  
 Glucose\*H<sub>2</sub>O: Applichem, A3730,0500, 500 g  
 CaCl<sub>2</sub>\*2H<sub>2</sub>O: Merck, 2382.1000, 1 kg  
 BDM (2,3-Butanedione monoxime): Sigma, B0753-1kg, 1kg  
 HEPES: Applichem, A1069,0500, 500 g  
 H<sub>2</sub>O: Aqua ad inj., B. Braun, 1 L, 3703452  
 Filtration unit: Steritop Quick Release, Millipore, S2GPT05RE

## **4% Agarose Gel** for embedding

Prepare slicing buffer, but omit glucose. Add 4 g low-melt Agarose (Agarose Low Melt, 100 g: Carl Roth, 6351.2) to 100 mL glucose-free slicing buffer. Dissolve at 80°C in a water bath (approx. 15min). Store at 4°C for months.

## **Medium 199** for cultivation

500 mL Medium 199 (Gibco, 31150-022) with addition of:  
 5 mL ITS-X-supplement (Gibco, 10 mL, 5150056)  
 5 mL Penicillin-Streptomycin x100 (Sigma, P0781-100ML)

Split into 50 mL portions (Falcon tubes, Corning, 352070) and store at 4°C.

Add 50 µM β-Mercaptoethanol (Applichem, A1108,100) to portions used within 7 days (add 1.8 µL β-ME to 0.5 mL of Medium 199, mix, add 1 µL/mL of dilution to final medium)

Medium exchange: Prewarm medium thoroughly (37°C), retain approx. 0.8 mL old medium in each dish, add 1.6 mL fresh medium.