APPLICATION NOTE AN-900L-02

Perfusion Buffers and Reagents

Langendorff System – Model 900L | Danish Myo Technology A/S

Document information	
Document number	AN-900L-02
Applies to	Langendorff System 900L, mouse and rat hearts
Revision	1.0
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Related documents	User Manual: Section 3.1 (Have ready) and Chapter 5 (Heart Mounting); AN-900L-01

1 Purpose and Scope

This note gives the recipe for modified Krebs–Henseleit (KH) perfusion buffer and lists the reagents used in the DMT example experiment on the 900L. It covers rat and mouse hearts.

> **NOTE** The recipes are working examples. They are not independently validated. Follow your laboratory's approved scientific, animal-use and safety protocols.

2 Reagents

Reagent	Use
Heparin in saline, 1000 IU/mL	Anticoagulant (Section 5)
Ketamine/xylazine, or pentobarbital	Anesthesia (Section 5)
Modified Krebs–Henseleit solution	Perfusion buffer (Section 3)
Carbogen (95% O_2, 5% CO_2)	Gassing the buffer
DMSO or another suitable solvent	Dissolving test compounds (Section 4)

3 Modified Krebs-Henseleit Buffer

3.1 Prepare the Stock Solutions

Prepare each salt as a separate stock solution in Milli-Q water, using the amounts in Table 1. Weigh the amount shown and make up to 1 L.

Table 1 – Stock solutions

Salt	Concentration	MW (g/mol)	Amount (g per liter)
NaCl	4 M	58.44	233.76
KCl	2 M	74.55	149.10
$MgCl_2 \cdot 6H_2O$	1 M	203.30	203.30
$NaH_2PO_4 \cdot 2H_2O$	1 M	156.01	156.01
$CaCl_2 \cdot 2H_2O$	1 M	147.02	147.02
Glucose·H_2O	1 M	198.17	198.17
$NaHCO_3$	1 M	84.01	84.01

Filter the stock solutions to remove small particles. All buffers must be filtered before use. DMT recommends a glass microfiber filter (Whatman) that retains 98% of particles larger than 1.6 µm. The water for the double-jacketed reservoir does not need to be filtered.

3.2 Make the 20× KH Stock

This recipe makes 2000 mL of 20× stock.

1. Add the volumes in Table 2 to a clean container.
2. Make up to 2000 mL with Milli-Q water and mix.
3. Filter the stock to remove small particles, and store it as your laboratory requires.

Table 2 – 20× KH stock, 2000 mL

Stock solution	Volume	Final concentration in 1× buffer
2 M KCl	80 mL	4 mM
4 M NaCl	1200 mL	120 mM
1 M $CaCl_2$	100 mL	2.5 mM
1 M $MgCl_2$	24 mL	0.6 mM
1 M NaH_2PO_4	24 mL	0.6 mM

3.3 Make the Working Solution

1. Add the volumes in Table 3 of 20× KH stock, 1 M $NaHCO_3$ and 1 M glucose to a clean container.
2. Make up to the final volume with Milli-Q water and mix.
3. Filter the solution (Section 3.1).
4. Bubble the solution with carbogen for at least 20 minutes.
5. Warm the buffer in the water bath at 37 °C and keep bubbling heavily until the end of the experiment.
6. Measure the pH before use. The pH must be 7.4.

Table 3 – Working solution volumes. Final $NaHCO_3$ is 25 mM and glucose 11 mM.

Final volume	20× KH stock	1 M $NaHCO_3$	1 M glucose
1 L	50 mL	25 mL	11 mL
2 L	100 mL	50 mL	22 mL
4 L	200 mL	100 mL	44 mL

3.4 How Much Buffer to Prepare

Heart	Buffer per experiment
Mouse	About 1 L
Rat	About 2–4 L, depending on how long the experiment lasts

Include the buffer for rinsing and for the pump line. Do not let the pump run dry.

4 Testing Compounds

Dissolve the compounds in DMSO or another appropriate solvent. Dilute them in the KH buffer for retrograde delivery to the heart.

> **NOTE** Keep the final solvent concentration as low as possible without affecting the solubility of the compound, and keep it the same in control and treated hearts.

5 Anesthesia and Anticoagulant

In the DMT example, rats are anesthetized with an intraperitoneal injection of ketamine/xylazine. Heparin is given intravenously (femoral vein) or intraperitoneally to prevent clots in the coronary vessels.

> **WARNING** Use only the agents, doses and routes in your approved animal-use protocol. DMT does not recommend doses. Dose, route and technique must be approved by your ethics committee.

The preparation and mounting of the heart are described in AN-900L-01.

6 Safety

- Carbogen is oxygen-enriched. Keep cylinders secured, use a regulator and keep flames and oil or grease away from fittings.
- DMSO passes through skin. Wear gloves.
- Handle needles and sharps according to your laboratory rules.
- Treat buffer that has passed through a heart as biological waste.