

# **Physiological buffer solutions: Extracellular and stock solutions**

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## **Table of Contents**

- 1. Introductory notes and guidelines**
- 2. "Recepies" for extracellular buffer solutions**

### **Stock Solutions**

CaCl<sub>2</sub> (0.1 M)  
NaCl (1.0 M)  
MgCl<sub>2</sub> (0.1 M)  
KCl (0.1 M)  
KCl (1.0 M)  
Hepes (1.0 M)  
EGTA (0.1 M)  
NaHCO<sub>3</sub> (0.1 M)  
MnCl<sub>2</sub> (0.1 M)  
MnCl<sub>2</sub> (100 μM)  
Na<sub>2</sub>HPO<sub>4</sub> (0.1 M)  
KH<sub>2</sub>PO<sub>4</sub> (0.1 M)  
BaCl<sub>2</sub> (0.1 M)

### **"Normal" physiological salt solutions**

~~2.5CaNa~~

### **"New" normal physiological salt solutions**

2CaNa

"Swiss Buffer"

Phosphate buffered solution, PBS

Krebs Henseleit Solution, KHS

Tris buffer

gangi2\science\_vcbl\lab stuff\extracell. buffers.01

Nr:Part I

**Ca<sup>2+</sup>-free solutions**

0CaNa

0CaNa, EGTA

0CaNa, high EGTA

0CaSwiss

0CaSwiss, EGTA

0CaPBS

0CaPBS, EGTA

0CaTris

**Ca<sup>2+</sup> substitutions**

2BaNa

10BaNa

20BaNa

**Na<sup>+</sup> substitutions**

2Ca19Na

0Ca19Na

0Ca19Na, EGTA

**High K<sup>+</sup> (depolarizing)**

2Ca30K

2Ca50K

2Ca70K

**“Super” High K<sup>+</sup> (depolarizing)**

2Ca130K+13Na

0Ca130K+13Na, EGTA

2Ca130K+0Na

0Ca130K+0Na, EGTA

**Cell storage and isolation media**

EH

EH-loading

EH-storage

**Cell isolation media**

Collagenase

Collagenase with trypsin inhibitor

**Empty sheet**

### **3. Elution buffers and Incubation buffers**

#### **eNOS Buffers**

#### **Glycation buffer**

#### **Glycogen II borate buffer**

Washing

Elution

## 2. Recipes

### Stock Solutions

Ions	Salt	MW	Conc. (M)	g for 1000 ml	Name/date
CaCl <sub>2</sub>	CaCl <sub>2</sub> x 2H <sub>2</sub> O	147.02	0.1	14.70	
NaCl	NaCl	58.44	1.0	58.44	
MgCl <sub>2</sub>	MgCl <sub>2</sub> x 6 H <sub>2</sub> O	203.31	0.1	20.33	
KCl	KCl	74.56	<u>0.1</u>	7.46	
KCl	KCl	74.56	<u>1.0</u>	74.6	
Hepes	free acid	238.31	1.0	238.31*	
EGTA	free acid	380.35	0.1	38.04*	
NaHCO <sub>3</sub>	NaHCO <sub>3</sub>	84.01	0.1	8.40	
MnCl <sub>2</sub>	MnCl <sub>2</sub> x 2 H <sub>2</sub> O	161.88	0.1	16.19	
MnCl <sub>2</sub>	MnCl <sub>2</sub>		<u>100 μM</u>	1 ml of 0.1 M	
Na <sub>2</sub> HPO <sub>4</sub>	Na <sub>2</sub> HPO <sub>4</sub> x2H <sub>2</sub> O	177.99	0.1	17.80	
KH <sub>2</sub> PO <sub>4</sub>			0.1		
BaCl <sub>2</sub>	BaCl <sub>2</sub> x 2 H <sub>2</sub> O	224.28	0.1	22.43	

\* preferable make a 100 ml of the stock solution (23.83 g Hepes acid for 100 ml)

# preferable make 10 ml of the stock solution (0.38 g EGTA for 10 ml)

#### NOTES:

1. Make these stock solutions to make the final buffers. Check routinely whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore and prepare new ones.

**Kontrolliere jede Woche ob die Stammlösungen kein "Flantschkerl" zeigen. Wenn Du welche findest, verwirf die Lösung und stelle eine neue Stammlösung her.**

2. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

**Wenn Du eine Lösung herstellst, verwende bitte eine Kopie als Vorlage und hake alle Komponenten, die Du zugibst ab. Gib Deinen Namen und das Datum an. Danach verwahre diese Kopie im Ordner, der im Labor aufliegt.**

3. Prepare the solutions as carefully as possible. Remember everybody is trusting you!

**Vergiß bitte nie, daß die anderen Dir vertrauen! Bemühe Dich alles richtig zu machen.**

**"Normal" physiological salt solutions 1****"New" normal physiological salt solutions 1**

2.5CaNa - 2CaNa - Swiss buffer

Components	2.5CaNa		2CaNa		"Swiss Buffer"	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	2.5	25 ml of 0.1 M	2	20 ml of 0.1 M	2	20 ml of 0.1 M
NaCl	140	140 ml of 1.0 M	138	138 ml of 1.0 M	130	130 ml of 1.0 M
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1	10 ml of 0.1 M	1	10 ml of 0.1 M
KCl	5	50 ml of 0.1 M	5	50 ml of 0.1 M	5.6	56 ml of 0.1 M
Hepes	10	10 ml of 1.0 M	10	10 ml of 1.0 M	8	8 ml of 1.0 M
NaHCO <sub>3</sub>	---	---	---	---	---	---
Glucose x 1 H <sub>2</sub> O	---	---	10	2.0 g	10	2.0 g
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M	7.45	~3-5 ml of 1.0 M
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

3. In terms of publication any of these solutions might be termed HBS (Hepes-buffered solution).

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

**Wenn Du eine Lösung herstellst, verwende bitte eine Kopie als Vorlage und hake alles ab wenn Du es Du zugibst. Gib Deinen Namen und das Datum an. Danach gib diese Kopie in den Laborordner.**

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**"Normal" physiological salt solutions 2**

PBS

KHS

Tris

Components	PBS		KHS		Tris	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	2	20 ml of 0.1 M	2.5	25 ml of 0.1 M	---	---
NaCl	137	137 ml of 1.0 M	118.4	118.4 ml of 1.0 M	---	---
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1.2	12 ml of 0.1 M	---	---
KCl	2.7	27 ml of 0.1 M	5.01	50.1 ml of 0.1 M	---	---
Na <sub>2</sub> HPO <sub>4</sub>	8	80 ml of 0.1 M	---	---	---	---
NaHCO <sub>3</sub>	---	---	25	25 ml of 1.0 M	---	---
KH <sub>2</sub> PO <sub>4</sub>	1.5	15 ml of 0.1 M	1.2	12 ml of 0.1 M	---	---
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M	---	---
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

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# "Ca<sup>2+</sup> free solutions" physiological salt solutions 1

0CaNa - 0CaNa, EGTA - 0CaNa, high EGTA

Components	0CaNa		0CaNa, EGTA		0CaNa, high EGTA	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	---	---	---	---	---	---
NaCl	138	138 ml of 1.0 M	138	138 ml of 1.0 M	138	138 ml of 1.0 M
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1	10 ml of 0.1 M	1	10 ml of 0.1 M
KCl	5	50 ml of 0.1 M	5	50 ml of 0.1 M	5	50 ml of 0.1 M
Hepes	10	10 ml of 1.0 M	10	10 ml of 1.0 M	10	10 ml of 1.0 M
EGTA	---	---	0.1	10 ml of 10 mM	1	100 ml of 10 mM
Glucose x 1 H <sub>2</sub> O	10	2.0 g	10	2.0 g	10	2.0 g
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M
Person, date						

## NOTES:

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

3. In terms of publication any of these solutions might be termed HBS (Hepes-buffered solution).

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

**Wenn Du eine Lösung herstellst, verwende bitte eine Kopie als Vorlage und hake alles ab wenn Du es Du zugibst. Gib Deinen Namen und das Datum an. Danach gib diese Kopie in den Laborordner.**

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**"Ca<sup>2+</sup> free solutions" physiological salt solutions 2**

0CaSwiss - 0CaSwiss, EGTA

		0CaSwiss		0CaSwiss, EGTA		
Components	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	---	---	---	---		
NaCl	130	130 ml of 1.0 M	130	130 ml of 1.0 M		
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1	10 ml of 0.1 M		
KCl	5.6	56 ml of 0.1 M	5.6	56 ml of 0.1 M		
Hepes	8	8 ml of 1.0 M	8	8 ml of 1.0 M		
EGTA	---	---	0.1	10 ml of 10 mM		
Glucose x 1 H <sub>2</sub> O	10	2.0 g	10	2.0 g		
pH (with NaOH)	7.45	~3-5 ml of 1.0 M	7.45	~3-5 ml of 1.0 M		
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

3. In terms of publication any of these solutions might be termed HBS (Hepes-buffered solution).

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

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**"Ca<sup>2+</sup> free solutions" physiological salt solutions 3**

0CaPBS - 0CaTris - 0CaPBS, EGTA

Components	0CaPBS		0CaTris		0CaPBS, EGTA	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	---	---	---	---	---	---
NaCl	137	137 ml of 1.0 M	---	---	137	137 ml of 1.0 M
MgCl <sub>2</sub>	1	10 ml of 0.1 M	---	---	1	10 ml of 0.1 M
KCl	2.7	27 ml of 0.1 M	---	---	2.7	27 ml of 0.1 M
Na <sub>2</sub> HPO <sub>4</sub>	8	80 ml of 0.1 M	---	---	8	8 ml of 1.0 M
EGTA	---	---	---	---	10 <sup>-4</sup>	10 ml of 10 mM
KH <sub>2</sub> PO <sub>4</sub>	1.5	15 ml of 0.1 M	--	--	1.5	15 ml of 0.1 M
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	---	---	7.4	~3-5 ml of 1.0 M
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

3. In terms of publication any of these solutions might be termed HBS (Hepes-buffered solution).

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

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**"Ca<sup>2+</sup> substitutions" 1**

2MnNa - 0.1MnNa - 200MnNa

Components	2MnNa		0.1MnNa		200MnNa	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
MnCl <sub>2</sub>	2	20 ml of 0.1 M	0.1	1 ml of 0.1 M	200 nM	2 ml of a 100 μM
NaCl	138	138 ml of 1.0 M	138	138 ml of 1.0 M	138	138 ml of 1.0 M
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1	10 ml of 0.1 M	1	10 ml of 0.1 M
KCl	5	50 ml of 0.1 M	5	50 ml of 0.1 M	50 ml	50 ml of 0.1 M
Hepes	10	10 ml of 1.0 M	10	10 ml of 1.0 M	10	10 ml of 1.0 M
EGTA	---	---	---	---	---	---
D-glucose x 1 H <sub>2</sub> O	10	2.0 g	10	2.0 g	10	2.0 g
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

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4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

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**"Ca<sup>2+</sup> substitutions" 2**

2BaNa - 10BaNa - 20BaNa

Components	2BaNa		10BaNa		20BaNa	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
BaCl <sub>2</sub>	2	20 ml of 0.1 M	10	100 ml of 0.1 M	20	200 ml of 0.1 M
NaCl	138	138 ml of 1.0 M	138	138 ml of 1.0 M	138	138 ml of 1.0 M
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1	10 ml of 0.1 M	1	10 ml of 0.1 M
KCl	5	50 ml of 0.1 M	5	50 ml of 0.1 M	50 ml	50 ml of 0.1 M
Hepes	10	10 ml of 1.0 M	10	10 ml of 1.0 M	10	10 ml of 1.0 M
EGTA	---	---	---	---	---	---
D-glucose x 1 H <sub>2</sub> O	10	2.0 g	10	2.0 g	10	2.0 g
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

3. In terms of publication any of these solutions might be termed HBS (Hepes-buffered solution).

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

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**"Na<sup>+</sup> substitutions" 1**

2Ca19Na - 0Ca19Na - 0Ca19Na, EGTA

Components	2Ca19Na		0Ca19Na		0Ca19Na, EGTA	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	2	20 ml of 0.1 M	---	---	---	---
NaCl	19	19 ml of 1.0 M	19	19 ml of 1.0 M	19	19 ml of 1.0 M
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1	10 ml of 0.1 M	1	10 ml of 0.1 M
KCl	5	50 ml of 0.1 M	5	50 ml of 0.1 M	5.6	56 ml of 0.1 M
Hepes	10	10 ml of 1.0 M	10	10 ml of 1.0 M	10	10 ml of 1.0 M
Choline chlorid	119	119 ml of 1.0 M	119	119 ml of 1.0 M	119	119 ml of 1.0 M
EGTA	---	---	---	---	10 <sup>-4</sup>	10 ml of 10 mM
Glucose x 1 H <sub>2</sub> O	10	2.0 g	10	2.0 g	10	2.0 g
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M	7.45	~3-5 ml of 1.0 M
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

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**High K<sup>+</sup>, depolarizing solutions 1**

2Ca30K - 2Ca50K - 2Ca70K

Components	2Ca30K		2Ca50K		2Ca70K	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	2	20 ml of 0.1 M	2	20 ml of 0.1 M	2	20 ml of 0.1 M
NaCl	113	113 ml of 1.0 M	93	93 ml of 1.0 M	73	73 ml of 1.0 M
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1	10 ml of 0.1 M	1	10 ml of 0.1 M
KCl	30	30 ml <u>of 1.0 M</u>	50	50 ml <u>of 1.0 M</u>	70	70 ml <u>of 1.0 M</u>
Hepes	10	10 ml of 1.0 M	10	10 ml of 1.0 M	8	8 ml of 1.0 M
NaHCO <sub>3</sub>	---	---	---	---	---	---
Glucose x 1 H <sub>2</sub> O	---	---	10	2.0 g	10	2.0 g
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M	7.45	~3-5 ml of 1.0 M
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

3. In terms of publication any of these solutions might be termed HBS (Hepes-buffered solution).

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

**Wenn Du eine Lösung herstellst, verwende bitte eine Kopie als Vorlage und hake alles ab wenn Du es Du zugibst. Gib Deinen Namen und das Datum an. Danach gib diese Kopie in den Laborordner.**

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**”Super” High K<sup>+</sup>, depolarizing solutions 2**

2Ca130K+13Na - 2Ca130K+0Na

Components	2Ca130K+13Na		---		2Ca130K+0Na	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	2	20 ml of 0.1 M	---	---	2	20 ml of 0.1 M
NaCl	13	13 ml of 1.0 M	---	---	---	---
MgCl <sub>2</sub>	1	10 ml of 0.1 M	---	---	1	10 ml of 0.1 M
KCl	130	130 ml of <u>1.0 M</u>	---	---	130	130 ml of <u>1.0 M</u>
Hepes	10	10 ml of 1.0 M	---	---	10	10 ml of 1.0 M
Choline chlorid	---	---	---	---	13	13 ml of 1.0 M
Glucose x 1 H <sub>2</sub> O	10	2.0 g	---	---	10	2.0 g
pH (with KOH)	7.4	~3-5 ml of 1.0 M	---	---	7.45	~3-5 ml of 1.0 M
Person, date						

**NOTES:**

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

3. In terms of publication any of these solutions might be termed HBS (Hepes-buffered solution).

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

**Wenn Du eine Lösung herstellst, verwende bitte eine Kopie als Vorlage und hake alles ab wenn Du es Du zugibst. Gib Deinen Namen und das Datum an. Danach gib diese Kopie in den Laborordner.**

5. Prepare the solutions as carefully as possible. Remember everybody is trusting you!

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## "Super" High K<sup>+</sup> & "Ca<sup>2+</sup>-free", depolarizing solutions

0Ca130K+13Na, EGTA - 0Ca130K+0Na, EGTA

0Ca130K+13Na, EGTA			---	0Ca130K+0Na, EGTA		
Components	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	---	---	---	---	---	---
NaCl	13	13 ml of 1.0 M	---	---	---	---
MgCl <sub>2</sub>	1	10 ml of 0.1 M	---	---	1	10 ml of 0.1 M
KCl	130	130 ml of 1.0 M	---	---	130	130 ml of 1.0 M
Hepes	10	10 ml of 1.0 M	---	---	10	10 ml of 1.0 M
Choline chlorid	---	---	---	---	13	13 ml of 1.0 M
EGTA	10 <sup>-4</sup>	10 ml of 10 mM	---	---	10 <sup>-4</sup>	10 ml of 10 mM
Glucose x 1 H <sub>2</sub> O	10	2.0 g	---	---	10	2.0 g
pH (with KOH)	7.4	~3-5 ml of 1.0 M	---	---	7.4	~3-5 ml of 1.0 M
Person, date						

### NOTES:

1. Use stock solutions to make final buffers. Check prior using them whether the stock solutions are clear or do have some bugs in it. If so do not use them anymore.

**Verwende bitte nur Stammlösungen ohne "Flantschkerl".**

2. Total ml NaOH added gives number of mM extra added to that added by NaCl (e.g. if you need 3 ml to set pH of 7.4 your final Na<sup>+</sup> concentration is 141 instead of 138 mM).

3. In terms of publication any of these solutions might be termed HBS (Hepes-buffered solution).

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

**Wenn Du eine Lösung herstellst, verwende bitte eine Kopie als Vorlage und hake alles ab wenn Du es Du zugibst. Gib Deinen Namen und das Datum an. Danach gib diese Kopie in den Laborordner.**

5. Prepare the solutions as carefully as possible. Remember everybody is trusting you!

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**Vessel storage, cell loading & storage media (NOT for cell culture)**

EH-vessel - EH-loading - EH-storage

NICHT für die Zellkultur

Components	EH-vessel		EH-loading		EH-storage	
	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>	2	20 ml of 0.1 M	2	20 ml of 0.1 M	2	20 ml of 0.1 M
NaCl	135	135 ml of 1.0 M	135	135 ml of 1.0 M	135	135 ml of 1.0 M
MgCl <sub>2</sub>	1	10 ml of 0.1 M	1	10 ml of 0.1 M	1	10 ml of 0.1 M
KCl	5	50 ml of 0.1 M	5	50 ml of 0.1 M	5	50 ml of 0.1 M
Hepes	20	20 ml of 1.0 M	<u>10</u>	10 ml of 1.0 M	<u>10</u>	10 ml of 1.0 M
NaHCO <sub>3</sub>	2.6	26 ml of 0.1 M	2.6	26 ml of 0.1 M	2.6	26 ml of 0.1 M
KH <sub>2</sub> PO <sub>4</sub>	0.44	4.4 ml of 0.1 M	0.44	4.4 ml of 0.1 M	0.44	4.4 ml of 0.1 M
Na <sub>2</sub> HPO <sub>4</sub>	0.34	3.4 ml of 0.1 M	0.34	3.4 ml of 0.1 M	0.34	3.4 ml of 0.1 M
Amino Acids	1X	20 ml of 50X	1X	20 ml of 50X	1X	20 ml of 50X
Vitamins	1X	10 ml of 100X	1X	10 ml of 100X	1X	10 ml of 100X
Phenol Red	0.001%	2 ml of 0.5%	---	---	---	---
Glucose x 1 H <sub>2</sub> O	10	2.0 g	10	2.0 g	10	2.0 g
L-Glutamine	2	367.2 mg	2	367.2 mg	2	367.2 mg
pH (HCl/NaOH)	7.35	~?? ml of 1.0 M	7.45	~3-5 ml of 1.0 M	7.45	~?? ml of 1.0 M
Penic./Strep Fungizone	1% 1%	10 ml 5 ml	1% 1%	10 ml 5 ml	1% 1%	10 ml 5 ml
horse serum (HS)	2 %	20 ml			2 %	20 ml
Person, date						

**Vessel storage, cell loading & storage media (NOT for cell culture)**EH-vessel - EH-loading - EH-storage **for 200 ml** NICHT für die Zellkultur

Components	EH-vessel		EH-loading/-storage		EH-storage	
	Conc. (mM)	200 ml total	Conc. (mM)	200 ml total	Conc. (mM)	200 ml total
CaCl <sub>2</sub>	2	4 ml of 0.1 M	2	4 ml of 0.1 M	2	4 ml of 0.1 M
NaCl	135	27 ml of 1.0 M	135	27 ml of 1.0 M	135	27 ml of 1.0 M
MgCl <sub>2</sub>	1	2 ml of 0.1 M	1	2 ml of 0.1 M	1	2 ml of 0.1 M
KCl	5	10 ml of 0.1 M	5	10 ml of 0.1 M	5	10 ml of 0.1 M
Hepes	20	4 ml of 1.0 M	<u>10</u>	2 ml of 1.0 M	<u>10</u>	2 ml of 1.0 M
NaHCO <sub>3</sub>	2.6	5.2 ml of 0.1 M	2.6	5.2 ml of 0.1 M	2.6	5.2 ml of 0.1 M
KH <sub>2</sub> PO <sub>4</sub>	0.44	0.88 ml of 0.1 M	0.44	0.88 ml of 0.1 M	0.44	0.88 ml of 0.1 M
Na <sub>2</sub> HPO <sub>4</sub>	0.34	0.68 ml of 0.1 M	0.34	0.68 ml of 0.1 M	0.34	0.68 ml of 0.1 M
Amino Acids	1X	4 ml of 50X	1X	4 ml of 50X	1X	4 ml of 50X
Vitamins	1X	2 ml of 100X	1X	2 ml of 100X	1X	2 ml of 100X
Phenol Red	0.001%	0.4 ml of 0.5%	---	---	---	---
Glucose x 1 H <sub>2</sub> O	10	0.2 g	10	0.2 g	10	0.2 g
L-Glutamine	2	367.2 mg	2	367.2 mg	2	367.2 mg
pH (HCl/NaOH)	7.35	~?? ml of 1.0 M	7.45	~0.6-1 ml of 1.0 M	7.45	~?? ml of 1.0 M
Penic./Strep	1%	2 ml	1%	2 ml	1%	2 ml
Fungizone	1%	1 ml	1%	1 ml	1%	1 ml
horse serum (HS)	2 %	4 ml			2 %	4 ml
Person, date						

NOTES:

1. The EH media is a type of Eagle's Minimum Essential Medium (EMEM) and the DMEM is somewhat similar to the Dulbeccos Minimum Essential Medium (DMEM), but also has HEPES as a pH buffer so that bubbling the solution with CO<sub>2</sub> is not necessary for maintenance of pH.

**Du brauchst die oben angeführten Lösungen nicht mit CO<sub>2</sub> durchperlen. Stelle den pH auf ca. 7.3 ein und filtriere dann die Lösung. Das erhöht den pH um ca. 0.15 Einheiten.**

2. The EH medium is best for storage of isolated and cleaned vessels from the slaughterhouse and in the fridge. The "DMEM, loading" is for loading the cells with the fluorescence dye at room temperature. The "DMEM, storage" should be used to save the cells until experiments (even equilibration time).

3. Adjust the pH prior the horse serum is added. Serum proteins will clog pH electrode.

**Stelle den pH vor der Zugabe des Pferdeserums (horse serum) ein.**

4. Use a copy of this recipe as a worksheet and check off components as added. Enter date and your name. Save this worksheet in the provided folder in the laboratory.

**Wenn Du eine Lösung herstellst, verwende bitte eine Kopie als Vorlage und hake alle Komponenten die Du zugibst ab. Gib Deinen Namen und das Datum an. Danach verwahre diese Kopie im Ordner der im Labor aufliegt.**

5. Prepare the solutions as carefully as possible. Remember everybody is trusting you!

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**Cell isolation media 1****(for cell isolation for culture and experiments)**

Collagenase Collagenase w. trypsin inhibitor

(Für Zellisolierungen für Zellkultur und Experimente)

Components	Collagenase		Collagenase, trypsin inh.		Conc. (mM)	1000 ml total
	Conc. (mM)	500 ml total	Conc. (mM)	500 ml total		
CaCl <sub>2</sub>	0.5	2.5 ml of 0.1 M	0.5	2.5 ml of 0.1 M		
Collagenase Type II	200 U/ml	270 mg	200 U/ml	270 mg		
Trypsin inhib.(II-S)	---	---	500 mg	1 mg/ml		
BSA	2 mg/ml	1000 mg	2 mg/ml	1000 mg		
Hepes	10	10 ml of 1.0 M	10	10 ml of 1.0 M	---	---
Amino acids essential	1X	10 ml of 50X	1X	10 ml of 50X		
Amino acids non essential	1X	5 ml of 100X	1X	5 ml of 100X		
Vitamins	1X	5 ml of 100X	1X	5 ml of 100X		
D-glucose	10	2.0 g	10	2.0 g		
pH (with NaOH)	7.3	~3-5 ml of 1.0 M	7.3	~3-5 ml of 1.0 M		
horse serum (HS)	5%	25 ml				
Person, date						

**NOTES:**

1. Bubbling the solution with CO<sub>2</sub> is not necessary for maintenance of pH.

**Du brauchst die oben angeführten Lösungen nicht mit CO<sub>2</sub> durchperlen. Stelle den pH auf ca. 7.3 ein und filtriere dann die Lösung. Das erhöht den pH um ca. 0.15 Einheiten.**

2. Adjust the pH prior the horse serum is added. Serum proteins will clog pH electrode.

**Stelle den pH vor der Zugabe des Pferdeserums (horse serum) ein.**

3. Use a copy of this recipe as a worksheet and check off components and save it in the provided folder.

**Wenn Du eine Lösung herstellst, bitte verwende eine Kopie als Vorlage und hake alle Komponenten, die Du zugibst ab. Gib Deinen Namen an und verwahre diese Kopie im Ordner, der im Labor aufliegt.**

4. Prepare the solutions as carefully as possible. Remember everybody is trusting you!

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**Empty sheet:**

Please indicate your buffer by using the lab-stock solutions. IF no lab-stock solution is available create one by yourself.

.....						
Components	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total	Conc. (mM)	1000 ml total
CaCl <sub>2</sub>						
NaCl						
MgCl <sub>2</sub>						
KCl						
Hepes						
NaHCO <sub>3</sub>						
Glucose x 1 H <sub>2</sub> O						
pH (with NaOH)	7.4	~3-5 ml of 1.0 M	7.4	~3-5 ml of 1.0 M	7.45	~3-5 ml of 1.0 M
Person, date						