

Krebs-Ringer's solution

Standard physiological buffer that is suitable for various calcium imaging experiments.

In a 1000 ml bottle, start with approx. 500 ml of dH₂O (double distilled) and add in following order while stirring.

| Substance | FW | 1X Weight | 10X Weight | Final (mM) |
|--|--------|-----------------|------------|------------|
| NaCl | 58.44 | 6.95 g | 69.54 g | 119.0 |
| KCl | 74.55 | 0.19 g | 1.86 g | 2.5 |
| NaH ₂ PO ₄ (monobasic) | 137.99 | 0.14 g | 1.38 g | 1.0 |
| CaCl ₂ ·2H ₂ O (Ca ²⁺ stock sol.) | 147.02 | 0.37 g (2.5 ml) | - | 2.5 |
| MgCl ₂ ·6H ₂ O (Mg ²⁺ stock sol.) | 203.30 | 0.26 g (1.3 ml) | - | 1.3 |
| HEPES | 238.30 | 4.77 g | 47.66 g | 20.0 |
| D-glucose (dextrose) C ₆ H ₁₂ O ₆ | 180.16 | 1.98 g | - | 11.0 |

Bring up to 1000 ml with dH₂O and adjust to pH 7.4 at 25°C with NaOH and HCl. Store at 4°C. Store 10X Krebs-Ringer's solution (in 50 ml aliquots) at 4°C. In a 500 ml bottle, add 50 ml 10X Krebs-Ringer's solution, 1.25 ml Ca²⁺ stock solution, 0.65 ml Mg²⁺ stock solution, plus 0.99 g D-glucose. Bring up to 500 ml and adjust to pH 7.4 at 25°C with NaOH and HCl. Store at 4°C.

Mac Ewen medium

For experiments using isolated smooth muscle cells (Nicolas Fritz). In a 1000 ml bottle, start with approx. 500 ml of dH₂O (double distilled) and add while stirring.

| Substance | FW | 1X Weight | 10X Weight | Final (mM) |
|--|--------|---------------|------------|------------|
| NaCl | 58.44 | 7.31 g | 73.05 g | 125.0 |
| KCl | 74.55 | 0.42 g | 4.17 g | 5.6 |
| CaCl ₂ ·2H ₂ O (Ca ²⁺ stock sol.) | 147.02 | 0.29 g (2 ml) | - | 2.0 |
| MgCl ₂ ·6H ₂ O (Mg ²⁺ stock sol.) | 203.30 | 0.20 g (1 ml) | - | 1.0 |
| HEPES | 238.30 | 1.91 g | 19.06 g | 8.0 |
| D-glucose (dextrose) C ₆ H ₁₂ O ₆ | 180.16 | 1.98 g | - | 11.0 |

Bring up to 1000 ml with dH₂O and adjust to pH 7.4 at 25°C with NaOH and HCl. Store at 4°C. Store 10X Mac Ewen medium (in 50 ml aliquots) at 4°C. In a 500 ml bottle, add 50 ml 10X Mac Ewen medium, 1 ml Ca²⁺ stock solution, 0.5 ml Mg²⁺ stock solution, plus 0.99 g D-glucose. Bring up to 500 ml and adjust to pH 7.4 at 25°C with NaOH and HCl. Store at 4°C.

Ca²⁺ and Mg²⁺ stock solutions

In two 100 ml bottles, start with approx. 50 ml of dH₂O and add in separate bottles while stirring.

| Substance | FW | Weight | Final (mM) |
|--------------------------------------|--------|---------|------------|
| CaCl ₂ ·2H ₂ O | 147.02 | 14.70 g | 1000.0 |
| MgCl ₂ ·6H ₂ O | 203.30 | 20.33 g | 1000.0 |

Bring up the two bottles to 100 ml with dH₂O and store at 4°C.

For Ca²⁺ free solutions add 2 mM EGTA (Sigma E4378) and omit the CaCl₂. Don't use EDTA which also buffers other divalent cations, such as Mg²⁺.

Artificial cerebrospinal fluid (ACSF)

ACSF 10X stock solution

In a 1000 ml bottle, start with approx. 500 ml of dH₂O (double distilled) and add in following order while stirring.

| Substance | FW | 1X Weight | 10X Weight | Final (mM) |
|--|--------|-----------|------------|------------|
| NaCl | 58.44 | 7.31 g | 73.05 g | 125.0 |
| NaHCO ₃ | 84.01 | 2.10 g | 21.00 g | 25.0 |
| NaH ₂ PO ₄ (monobasic) | 137.99 | 0.17 g | 1.72 g | 1.25 |
| D-glucose (dextrose) C ₆ H ₁₂ O ₆ | 180.16 | 3.60 g | 36.03 g | 20.0 |
| KCl | 74.55 | 0.37 g | 3.73 g | 5.0 |

Bring up to 1000 ml with dH₂O and store at 4°C. To the 1X ACSF add 2 ml Ca²⁺ stock solution and 1 ml Mg²⁺ stock solution before bringing up the volume to 1000 ml.

Store 10X ACSF in 50 ml aliquots at 4°C.

Ca²⁺ and Mg²⁺ stock solutions

In two 100 ml bottles, start with approx. 50 ml of dH₂O and add in separate bottles while stirring.

| Substance | FW | Weight | Final (mM) |
|--------------------------------------|--------|---------|------------|
| CaCl ₂ ·2H ₂ O | 147.02 | 14.70 g | 1000.0 |
| MgSO ₄ ·7H ₂ O | 246.48 | 24.65 g | 1000.0 |

Bring up the two bottles to 100 ml with dH₂O and store at 4°C.

ACSF solution

Dilute ACSF 10X stock solution ten times in dH₂O. Add proper volumes of Ca²⁺ and Mg²⁺ stock solutions. Standard concentrations are 2 mM Ca²⁺ and 1 mM Mg²⁺. For Ca²⁺ free solution add 2 mM EGTA (Sigma E4378). Don't use EDTA which buffers all divalent cations. For increased activity in NMDA-experiments use ACSF Mg²⁺-free. To get 500 ml of ACSF mix following:

| Solution | ACSF | ACSF Ca ²⁺ -free | ACSF Mg ²⁺ -free |
|---------------------------------|----------|-----------------------------|-----------------------------|
| dH ₂ O | 448.5 ml | 449.5 ml | 449.0 ml |
| ACSF 10X | 50.0 ml | 50.0 ml | 50.0 ml |
| Ca ²⁺ stock solution | 1.0 ml | | 1.0 ml |
| Mg ²⁺ stock solution | 0.5 ml | 0.5 ml | |
| EGTA (MW 380.35) | | 0.380 g | |

Add the Ca²⁺ stock solution dropwise and bubble with 95% O₂ / 5% CO₂ to avoid Ca²⁺ precipitation. Adjust pH to 7.3 or 7.4 for experiments in room temperature or in 37°C respectively (osmolarity ~300 mOsm). Keep the ACSF-solution bubbling with 95% O₂ / 5% CO₂ during the experiment.

For depolarization experiments exchange 50 mM of NaCl with 50 mM of KCl in the ACSF.

Cutting solution

Brain is sliced in ice-cold (≤4°C) regular ACSF or cutting-ACSF containing 8 mM Mg²⁺ and 1 mM Ca²⁺. A block of 2% agar can also be glued in the dish with the brain and positioned up against the tissue, antidiagonally to the blade, to stabilize the brain during slicing.