



Selected media and chemicals for respirometry with mitochondrial preparations

Fontana-Ayoub M, Fasching M, Gnaiger E

OROBOROS INSTRUMENTS Corp., high-resolution respirometry
Schöpfstr. 18, A-6020 Innsbruck, Austria
erich.gnaiger@oroboros.at; www.oroboros.at

Summary: Different media for tissue preparation and respiration are used in investigations of mitochondrial function. Initial decisions on the composition of media and chemicals are decisive for long-term studies and crucial for comparability of results. As a guideline, we summarize an update of our experience with media and chemicals for high-resolution respirometry with isolated mitochondria, permeabilized cells, muscle fibres and tissue homogenates. Whereas optimization is necessary for specific experimental protocols, standardization will improve the comparability of results obtained in different laboratories. Such efforts towards standardization are important for the advancement of mitochondrial physiology and mitochondrial medicine.

Contents

| | |
|---|---|
| 1. Introduction | 2 |
| 2. Media for muscle fibre preparation and isolation of mitochondria | 2 |
| 2.1. BIOPS for preparation of permeabilized muscle fibres..... | 2 |
| 2.2. Mitochondrial Preservation Medium: MiP03 | 3 |
| 2.3. Isolation of mitochondria from liver and placenta..... | 4 |
| 2.4. Isolation of mitochondria from skeletal muscle | 4 |
| 2.5. Isolation of mitochondria from heart | 5 |
| 3. Mitochondrial respiration media (MiR) | 5 |
| 3.1. MiR05(Cr), MiR06(Cr)..... | 5 |
| 3.2. Oxygraph medium for cytochrome c test | 5 |
| 4. Chemicals for mitochondrial SUIIT protocols | 5 |
| 4.1. Substrates for SUIIT protocols..... | 6 |
| 4.2. Uncouplers for SUIIT protocols | 7 |
| 4.3. Inhibitors for SUIIT protocols | 7 |
| 4.4. Agents for cell permeabilization | 8 |
| 5. General comments | 9 |
| 6. References | 9 |

1. Introduction

High-resolution respirometry provides the basis for a detailed analysis of mitochondrial function (OXPHOS analysis). Incubation media contain compounds such as sucrose, mannitol, potassium chloride, potassium-MES, to achieve physiological osmolarity. Additional components are added to preserve mitochondrial integrity. Mitochondrial media, therefore, have different ionic strengths, pH and ionic compositions.

The list of **media** is organized according to the major applications, including isolation of mitochondria, preparation of muscle fibres and incubation media for respirometry, with emphasis on **MiR06** (MiR06 = MiR05+Catalase; [MiPNet14.13](#)) as our most advanced respiration medium. The list of **chemicals** contains mitochondrial substrates, inhibitors, uncouplers and agents for cell permeabilization. The preferred concentrations and solvents are shown for stock solutions, and storage conditions are recommended.

Finding a compromise between dynamic optimization of SUIT protocols and adherence to a fixed standard represents a well-known problem in the development and application of strategies for scientific investigation. Improvement of standard methods requires cooperation and feedback. Therefore we appreciate any comments and suggestions directed towards improved and more generally acceptable standards in mitochondrial physiology.

2. Media for muscle fibre preparation and isolation of mitochondria

Higher respiratory capacities are observed when integrating a preservation strategy in the formulation of isolation media (such as addition of antioxidants). Improvement of the quality of isolation media may be limited by the increasing cost when preparing large volumes. The media for isolation of mitochondria ([Section 2.2](#) and [2.3](#)) are minimum media without concerns on preservation strategies.

2.1. BIOPS for preparation of permeabilized muscle fibres

(Veksler et al 1987; Letellier et al 1992)

The relaxing and biopsy preservation solution BIOPS contains 10 mM Ca-EGTA buffer, 0.1 μ M free calcium, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM $MgCl_2$, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.1.

BIOPS

Total volume = 1 litre

| | Final conc. | FW | Stock solution | Addition to 1 litre final | Source and product code |
|---------------------------------------|-------------|-------|----------------|---------------------------|-------------------------|
| CaK ₂ EGTA* | 2.77 mM | | 100 mM | 27.7 ml | |
| K ₂ EGTA* | 7.23 mM | | 100 mM | 72.3 ml | |
| Na ₂ ATP | 5.77 mM | 551.1 | | 3.141 g | Sigma A 2383, -20 °C |
| MgCl ₂ ·6 H ₂ O | 6.56 mM | 203.3 | | 1.334 g | Scharlau MA 0036, RT |

| | | | | | |
|---------------------------------|--------|--------|--|---------|-----------------------|
| Taurine | 20 mM | 125.1 | | 2.502 g | Sigma T 0625, RT |
| Na ₂ Phosphocreatine | 15 mM | 255.1* | | 4.097 g | Sigma P 7936, - 20 °C |
| Imidazole | 20 mM | 68.1 | | 1.362 g | Fluka 56750, RT |
| Dithiothreitol (DTT) | 0.5 mM | 154.2 | | 0.077 g | Sigma D 0632, 4 °C |
| MES | 50 mM | 195.2 | | 9.76 g | Sigma M8250, RT |

*Anhydrous

BIOPS contains the following ion concentrations:

| | | |
|-----------------------|--------|--|
| Ca ²⁺ free | 0.1 µM | Adjust the pH to 7.1 (with 5 N KOH) at 0 °C. Divide into 20 ml portions. Store BIOPS and K ₂ EGTA / CaK ₂ EGTA solutions at -20 °C in plastic vials. |
| Mg ²⁺ free | 1 mM | |
| MgATP | 5 mM | |
| Ionic strength | 160 mM | |

Preparation of stock solutions K₂EGTA and CaK₂EGTA:

K₂EGTA Mix 100 mM EGTA (Sigma, E 4378, 25 g) and 200 mM KOH (Sigma, P 1767, 1 kg) (dissolve 7.608 g EGTA and 2.3 g KOH in 200 ml H₂O, adjust the pH to c. 7.0 with KOH).

CaK₂EGTA Dissolve 2.002 g CaCO₃ (Sigma, C 4830; 100g) in 100 mM hot (80 °C) solution of EGTA (7.608 g / 200 ml) while stirring continuously, add 2.3 g KOH, adjust the pH to c. 7.0.

KH₂PO₄ ATP will be hydrolyzed at least partially during fibre storage, thus generating mM levels of inorganic phosphate. It has not been reported if addition of 3 mM phosphate (Veksler et al 1987; Skladal et al 1994) exerts any effect on preservation quality.

Saponin solution: for muscle permeabilization, prepared fresh everyday:

1. Saponin stock solution: add 5 mg saponin (Sigma S 2149; 25 g) to 1 ml BIOPS.
2. For permeabilization in saponin solution, add 20 µl saponin stock solution to 2 ml BIOPS.

2.2. Mitochondrial Preservation Medium: MiP03

Use MiR06 ([MiPNet14.13 Medium-MiR06](#)) and add the following:

| Compound | Final conc. | MW | Addition to 20 ml final volume | Company, product code and storage |
|---------------------|-------------|-------|--------------------------------|-----------------------------------|
| Histidine | 20 mM | 155.2 | 62.1 mg | Sigma, RT |
| Vitamin E succinate | 20 µM | 530.8 | 200 µl (2 mM stock) | Sigma, RT |
| Glutathion | 3 mM | 307.3 | 18.4 mg | Sigma, 4 °C |
| Leupeptine | 1 µM | 463.0 | 20 µl (1 mM stock) | Sigma, -20 °C |
| Glutamate | 2 mM | 169.1 | 40 µl (1 M stock) | Sigma, RT |
| Malate | 2 mM | 134.1 | 40 µl (1 M stock) | Sigma, RT |
| Mg-ATP | 2 mM | 614.1 | 80 µl (500 mM stock) | Sigma, -20 °C |

MiP03 preservation medium has the following final concentrations:

| | |
|-----------------------|----------|
| Ca ²⁺ free | 0.0 μM |
| Mg ²⁺ free | 2.1 mM |
| K ⁺ | 90 mM |
| Na ⁺ | 4 mM |
| EGTA free | 0.46 mM |
| Osmolarity | 340 mosM |
| Ionic strength | 108 mM |

Adjust the pH to 7.1 (5 N KOH) at 30 °C.

Vitamin E D-α-Tocopherol succinate is soluble in chloroform (50 mg/ml) or ethanol, it is practically insoluble in water and it is unstable in alkaline conditions. Solutions of D-α-Tocopherol are stable at 4 °C (light protected) for several months. 20 μM intracellular concentration in liver.

Leupeptine Soluble in water. The aqueous solution is stable for a week at 4 °C and for at least 6 months as frozen aliquots at -20 °C.

Storage Store 40 ml aliquots at -20 °C.

2.3. Isolation of mitochondria from liver and placenta

Medium A1 Total volume 1 litre

| | Final conc. | FW | Addition to 1 litre final volume |
|----------------------|-------------|-------|----------------------------------|
| Sucrose | 250 mM | 342.3 | 85.6 g |
| Na ₂ EDTA | 0.5 mM | 372.2 | 0.186 g |
| Tris | 10 mM | 121.1 | 1.211 g |

Adjust the pH to 7.4 (HCl) at c. 0 °C. Store at -20 °C in 100-200 ml plastic vials.

Medium B1: take 500 ml of medium A1 and add:

| | | | |
|-----|-------|--|--------------|
| BSA | 1 g/l | | 0.5 g/500 ml |
|-----|-------|--|--------------|

Store at -20°C in 100-200 ml plastic vials.

2.4. Isolation of mitochondria from skeletal muscle

Medium A2 Total volume 1 litre

| | Final conc. | FW | Addition to 1 litre final volume |
|----------------------|-------------|-------|----------------------------------|
| KCl | 180 mM | 74.55 | 13.42 g |
| Na ₂ EDTA | 0.5 mM | 372.2 | 0.186 g |
| Tris | 10 mM | 121.1 | 1.211 g |

Adjust the pH to 7.4 (HCl) at c. 0 °C. Store at -20 °C in plastic vials.

Medium B2: take 500 ml of medium A2 and add:

| | | | |
|-----|-------|--|--------------|
| BSA | 1 g/l | | 0.5 g/500 ml |
|-----|-------|--|--------------|

Store at -20 °C in plastic vials.

2.5. Isolation of mitochondria from heart

| Stock solution | Conc. | FW | Addition to 1 litre final volume |
|----------------|-------|--------|----------------------------------|
| D-Mannitol | 0.5 M | 182.17 | 91.085 g |
| Sucrose | 0.5 M | 342.30 | 171.150 g |
| EGTA* | 0.1 M | 380.35 | 38.350 g |

*Neutralize with Tris to pH 7.4

Isolation Medium

| | Final conc. | Addition to 200 ml final volume |
|--------------|-------------|---------------------------------|
| D-Mannitol | 225 mM | 90 ml |
| Sucrose | 75 mM | 30 ml |
| EGTA, pH 7.4 | 1 mM | 2 ml |

Prepare fresh daily and keep at 4 °C.

3. Mitochondrial respiration media (MiR)

» www.bioblast.at/index.php/List_of_media_for_respirometry

3.1. MiR05(Cr), MiR06(Cr)

MiR05 (Gnaiger et al 2000).

MiR06(Cr) = MiR05(Cr) + Catalase: see separate protocol ([MiPNet14.13 Medium-MiR06](#)).

3.2. Oxygraph medium for cytochrome c test

The high concentration of KCl favours dissociation of cytochrome *c* from the inner mitochondrial membrane and cytochrome *c* release upon injury of the outer mitochondrial membrane. Respiratory flux is reduced with cytochrome *c* depletion, and can be restored after addition of 10 µM cytochrome *c* (Saks et al 1992, 1995; Gnaiger and Kuznetsov 2002; Kuznetsov et al 2004).

| | Final conc. | FW | Addition to 1 litre final |
|---------------------------------------|-------------|-------|---------------------------|
| EGTA | 0.4 mM | 336.2 | 0.134 g |
| MgCl ₂ .6 H ₂ O | 3 mM | 203.3 | 0.61 g |
| KH ₂ PO ₄ | 5 mM | 136.1 | 0.68 g |
| Dithiothreitol | 0.3 mM | 154.2 | 0.046 g |
| KCl | 125 mM | 74.55 | 9.32 g |
| HEPES | 20 mM | 238.3 | 4.77 g |

Cytochrome c medium contains the following ion concentrations:

| | |
|-----------------------|---------|
| Ca ²⁺ free | 0.0 µM |
| Mg ²⁺ free | 2.51 mM |
| EGTA free | 0.36 µM |
| Ionic strength | 142 mM |

Adjust the pH to 7.1 (5 N KOH) at 25 °C. Divide into 20 ml portions. Store at -20 °C in plastic vials.

4. Chemicals for mitochondrial SUIIT protocols

Calculation of concentrations: [MiPNet09.12_O2k-Titrations.xls](#).

4.1. Substrates for SUIIT protocols

» www.bioblast.at/index.php/List_of_substrates_and_metabolites

| Substrate | FW | Stock soln. Conc [mM] | Stock Soln. Amount | Comments | Source, product code and storage |
|---|--------|-----------------------|---------------------------------|---|--|
| G: L-Glutamic acid, sodium salt, C ₅ H ₈ NO ₄ Na | 169.1 | 2000 | 3.382 g/ 10 ml H ₂ O | Neutralize with 5 N KOH, check pH. Divide into 0.5 ml portions. Store at -20 °C. | Sigma, G 1626, RT |
| M: L-Malic acid, C ₄ H ₆ O ₅ | 134.1 | 400 | 536 mg/ 10 ml H ₂ O | Neutralize with 10 N KOH, check pH. Divide into 0.5 ml portions. Store at -20°C. | Sigma, M 1000, RT |
| P: Pyruvic acid sodium salt, C ₃ H ₃ O ₃ Na | 110.0 | 2000 | 44 mg/ 0.2 ml H ₂ O | Prepare everyday fresh. | Sigma, P 2256, 4°C |
| S: Succinate disodium salt, hexahydrate, C ₄ H ₄ O ₄ Na ₂ x 6 H ₂ O | 270.1 | 1000 | 2.701 g/ 10 ml H ₂ O | Check pH and adjust if necessary to 7.0 with 1 N HCl. Divide into 0.5 ml portions. Store at -20 °C. | Sigma, S 2378, RT |
| Oct: DL-Octanoyl-carnitine-HCl, C ₁₅ H ₃₀ NO ₄ Cl | 323.85 | 100 | 32.4 mg/ ml H ₂ O | Store at -20 °C. | TOCRIS Bioscience, No. 0605, RT, desiccate |
| Pal: Palmitoyl-DL-carnitine-HCl, C ₂₃ H ₄₅ NO ₄ ·HCl | 436.1 | 10 | 8.72 mg/ 2 ml H ₂ O | Store at -20 °C. | Sigma P 4509, -20 °C |
| As: Ascorbate sodium salt, C ₆ H ₇ O ₆ Na | 198.1 | 800 | 1.584 g/ 10 ml H ₂ O | To prevent autooxidation, adjust pH to ~ 6 with ascorbic acid (a 137.6 mg ml ⁻¹ solution of pH ~ 2). Divide into 0.2 ml portions. Store at -20 °C. Light sensitive. | Sigma, A4034, RT |
| Tm: TMPD N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride, C ₁₀ H ₁₆ N ₂ ·2 HCl | 237.2 | 200 | 47.4 mg/ ml H ₂ O | To prevent autooxidation add 0.8 M ascorbate to a final concentration of 10 mM. Divide into 0.2 ml portions. Store at -20 °C. | Sigma, T3134, RT |
| c: Cytochrome c | 12500 | 4.0 | 50 mg/ ml H ₂ O | Divide into 0.2 ml portions. Store at -20 °C. | Sigma, C7752, -20°C |
| D: ADP** (Adenosine 5'diphosphate, C ₁₀ H ₁₅ N ₅ O ₁₀ P ₂ K, potassium salt, contains 1 mol/mol H ₂ O) | 501.3 | 500 | 0.501 g/ 2 ml H ₂ O | Neutralize with 5 N KOH (approx.450 µl), check pH. Divide into 0.2 ml portions. Store at -80 °C. ** To keep [Mg ²⁺] constant during respiration measurement mix ADP with MgCl ₂ (0.6 mol/mol ADP) | Cal-biochem, 216201, 4°C |

| | | | | | |
|---|--|-----|----------------------------|---|------------------------------------|
| T: ATP** (Adenosine 5'-triphosphate, $C_{10}H_{14}N_5O_{13}P_3Na_2$, disodium salt, contains 3.5 mol/mol H_2O) | 614.1 3.5 mol/ mol H_2O 551.1 anhy- drous | 500 | 0.614 g/ 2 ml H_2O | Neutralize with 5 N KOH (approx. 400 μ l), check pH. Divide into 0.2 ml portions. Store at $-80^\circ C$. ** To keep $[Mg^{2+}]$ constant during respiration measurement mix ATP with $MgCl_2$ (0.8 mol/mol ATP). | Sigma, A 2383, $-20^\circ C$ |
|---|--|-----|----------------------------|---|------------------------------------|

4.2. Uncouplers for SUIT protocols

» www.bioblast.at/index.php/List_of_uncouplers

| Uncoupler | FW | Stock soln. Conc. [mM] | Stock soln. Amount | Comments | Source, product code and storage |
|---|--------|------------------------|---------------------------|--|----------------------------------|
| U CCCP: $C_9H_5ClN_4$ | 204.62 | 1.0 | 1.02 mg in 5 ml ethanol | Store at $-20^\circ C$ | Sigma C 2759 |
| DNP: 2,4-Dinitrophenol, $C_6H_4O_5N_2$ | 184.1 | 10 | 3.7 mg/ 2 ml H_2O | Neutralize with 1 N KOH, check pH. Store at $-20^\circ C$. Toxic. | |
| F (FCCP): Carbonyl cyanide p-(trifluoro-methoxy) phenyl-hydrazone $C_{10}H_5F_3N_4O$ | 254.2 | 1.0 | 2.54 mg/ 10 ml ethanol | Divide into 0.5 ml portions. Store in glass vials at $-20^\circ C$. | Sigma, C 2920, $4^\circ C$ |
| TTFB: 4,5,6,7-Tetrachloro-2-trifluoromethyl-benzimidazole | 323.94 | 1.0 | 3.24 mg/ 10 ml ethanol | Divide into 0.5 ml portions. Store at $-20^\circ C$. | |

4.3. Inhibitors for SUIT protocols

» www.bioblast.at/index.php/List_of_inhibitors

| Inhibitor | FW | Stock soln. Conc. [mM] | Stock soln. Amount | Comments | Source, product code and storage |
|--|-------|------------------------|----------------------------------|---|--|
| Ama: Antimycin A | 540 | 5.0 | 11 mg/ 4 ml ethanol | Divide into 0.2 ml portions. Store at $-20^\circ C$. Very toxic. | Sigma, A 8674, $-20^\circ C$ |
| Amy: Amytal (Amobarbital) sodium salt, $C_{11}H_{17}N_2O_3Na$ | 248.3 | 200 | 0.497 g/ 10 ml 50% ethanol | Divide into 0.5 ml portions. Store at $-20^\circ C$. Light sensitive. Toxic. | |
| Atr: Atractyloside dipotassium salt, $C_{30}H_{44}O_{16}S_2K_2$ (2.5 mol/mol H_2O) | 803.0 | 50 | 40.2 mg/ 1 ml H_2O | Dissolves better in warm water. Store at $-20^\circ C$. Toxic. | Sigma, A 6882, RT |
| Azd: Sodium azide, NaN_3 | 65.01 | 4000 | 260 mg/ 1 ml H_2O | Divide into 0.5 ml portions. Store at $-20^\circ C$. Very toxic. | Sigma, S 2002, RT |
| Cat: Carboxy-atractyloside, potassium salt | 939.1 | 5 | 4.7 mg/ 1 ml H_2O | Divide into 0.2 ml portions. Store at $-20^\circ C$. Toxic. | Calbiochem 216201, $-20^\circ C$ |

| | | | | | |
|--|------------------------------|------------------|---------------------------------------|---|---------------------------|
| Kcn: Potassium cyanide, KCN | 65.12 | 1000 | 13 mg/ 0.2 ml H ₂ O | Prepare everyday fresh. The pH of the solution may be very alkaline; adjust with HCl. Photosensitive. Hygroscopic. Very toxic. | Fluka, 60178 |
| Mna: Malonic acid | 104.06 | 2000 | 0.0208 g/ 100 µl | Dissolve in 75 µl 5 N KOH, check pH, titrate small amounts (2 µl) of 5 N KOH until you reach a pH of 6.0, add H ₂ O to 100 µl. Prepare fresh | Sigma Aldrich, M129-6, RT |
| Myx: Myxothiazol | 487.7 | 1.0 | 1.0 mg/ 2.05 ml ethanol | Divide into 0.2 ml portions. Store at -20 °C. Very toxic. | Sigma, T-5580, 4°C |
| Omy: Oligomycin | 800 | 4 mg/ml =5 mM | 4 mg/ 1 ml ethanol | Divide into 0.2 ml portions. Store at -20 °C. Very toxic. | Sigma, O 4876, -20 °C |
| Oua: Ouabain (G-Strophanthin) octahydrate, C ₂₉ H ₄₄ O ₁₂ ·8 H ₂ O | 728.8 | 10 | 7.3 mg/ 1 ml H ₂ O | Divide into 0.2 ml portions. Store at -20 °C. Light sensitive. Toxic. | |
| Pep: p5-Di (adenosine -5') penta-phosphate sodium salt, C ₂₀ H ₂₉ N ₁₀ O ₂₂ P ₅ (5 mol/mol Na, 1.5 mol/mol H ₂ O) | 1058.4 916.4 free acid | 50 | 52.91 mg/ 1 ml H ₂ O | Neutralize with 5 N KOH, check pH. Divide into 0.2 ml portions. Store at -20 °C. Toxic. | |
| Rot: Rotenone, C ₂₃ H ₂₂ O ₆ | 394.4 | 1.0 ^a | 3.94 mg/ 10 ml ethanol | Difficult to dissolve. Store at -20 °C. Light sensitive. Very toxic. | Sigma R 8875 RT |
| Rut: Ruthenium red (ammoniated ruthenium oxychloride) | 551.22 | 10 | 5.5 mg/ 1 ml H ₂ O | Store at -20 °C. | |

^a Rotenone is added at a high final concentration (0.5 µM), based on a 1.0 mM stock solution. Since 0.1 µM may be fully inhibiting some mitochondrial preparations, a lower concentration may be used (0.2 mM stock, 0.1 µM final), to reduce the problem of rotenone retention in the O2k-chamber.

4.4. Agents for cell permeabilization

» www.bioblast.at/index.php/List_of_permeabilization_agents

| Substance | FW | Stock sol. Conc. | Stock solution Amount | Comments | Source, product code and storage |
|-----------------------|--------|------------------|-----------------------|-------------------------|----------------------------------|
| Dig: Digitonin | 1229.3 | 8.1 mM | 10 mg/1 ml DMSO | Store at -20 °C. Toxic. | Fluka, 37008, RT |
| Sap: Saponin | - | 5 mg/ml | 5 mg/1 ml BIOPS | Prepare fresh everyday. | Sigma, S7900, RT |

5. General comments

- 5.1. Solutions stored at low temperature: Mix carefully after re-warming, since phase separation may occur and compounds may precipitate in cold solutions. During the course of the experiment, keep stock solutions on ice.
- 5.2. Solutions containing ethanol: there may be a problem of evaporation and subsequent increase of concentration of stock solutions.
- 5.3. Chemicals dissolved in ethanol or DMSO: To check the influence of ethanol or DMSO on mitochondrial function and experimental sensors (ion selective electrodes), the same additions of pure solvents should be used in carrier control experiments.
- 5.4. For all stock solutions of mitochondrial substrates, inhibitors, and uncouplers; the total volumes of solutions are indicated.
- 5.5. Store chemicals as indicated by the suppliers. The storage conditions of prepared solutions are indicated in the comments.
- 5.6. Aliquots of stocks for rotenone, succinate, glutamate, malate, and oligomycin can be refrozen for later use, since these chemicals are stable.

6. References

- Gnaiger E, Kuznetsov AV (2002) Mitochondrial respiration at low levels of oxygen and cytochrome c. *Biochem Soc Trans* 30: 242-248.
- Gnaiger E, Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Steurer W, Margreiter R (2000) Mitochondria in the cold. In: *Life in the Cold*. (Heldmaier G, Klingenspor M, eds) Springer, Heidelberg, Berlin, New York: pp 431-442.
- Letellier T, Malgat M, Coquet M, Moretto B, Parrot-Roulaud F, Mazat J-P (1992) Mitochondrial myopathy studies on permeabilized muscle fibres. *Pediatr Res* 32: 17-22.
- Kuznetsov AV, Schneeberger S, Seiler R, Brandacher G, Mark W, Steurer W, Saks V, Usson Y, Margreiter R, Gnaiger E (2004) Mitochondrial defects and heterogeneous cytochrome c release after cardiac cold ischemia and reperfusion. *Am J Physiol Heart Circ Physiol* 286: H1633-H1641.
- Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopsies of human muscle. *Methods Mol Biol* 810: 25-58.
- Saks VA, Belikova YuO, Vasilleva EV, Kuznetsov AV, Lyapina S, Petrova L, Perov NA (1993) Retarded diffusion of ADP in cardiomyocytes: possible role of mitochondrial outer membrane and creatine kinase in cellular regulation of oxidative phosphorylation. *Biochim Biophys Acta* 1144: 134-148.
- Saks VA, Kuznetsov AV, Khuchua ZA, Vasileva EV, Belikova YO, Kesvatera T, Tiivel T (1995) Control of cellular respiration by mitochondrial outer membrane and by creatine kinase in normal muscle and in pathology. *J Mol Cell Cardiol* 27: 625-645.
- Skladal D, Sperl W, Schranzhofer R, Krismer M, Gnaiger E, Margreiter R, Gellerich FN (1994) Preservation of mitochondrial functions in human skeletal muscle during storage in high energy preservation solution (HEPS). In: *What is Controlling Life?* (Gnaiger E, Gellerich FN, Wyss M, eds) [Modern Trends in BioThermoKinetics 3. Innsbruck Univ. Press: 268-271.](#)
- Veksler VI, Kuznetsov AV, Sharov VG, Kapelko VI, Saks A (1987) Mitochondrial respiratory parameters in cardiac tissue: a novel method of assessment by using saponin-skinned fibres. *Biochim Biophys Acta* 892: 191-196.