MiR05-Kit instruction sheet

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Mitochondrial respiration medium: MiR05-Kit

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1. Composition

1.1 MiR05-Kit

MiR05-Kit [1 vial]	Formula weight [g/mol]	Amount for 250 mL final volume [g]	Final concentration [mM]
EGTA	380.4	0.047	0.5
$MgCl_2$	95.2	0.071	3
Lactobionic acid	358.3 free acid	5.375	60
Taurine	125.1	0.625	20
KH_2PO_4	136.1	0.340	10
HEPES	238.3	1.191	20
D-Sucrose	342.3	9.413	110

1.2 BSA to be added at the preparation

Substance	Final conc.	Addition to 250 mL final volume [g]	Source; storage temp.	Comment
BSA, fatty acid free	1 g/L	0.25	Sigma A 6003 fraction V (25 g); 4 °C	Not provided in the MiR05-Kit

2. Qualified personnel

The MiR05-Kit is a carefully mixed powder of solid chemicals. The powder is not to be partitioned by the user.

The mixture is not classified as hazardous. Nevertheless, the MiR05-Kit can be hazardous to untrained persons if handled inappropriately and not in accordance with the regulations of the preparation instructions. This instruction sheet provides precise safety indications to avoid hazards to all persons using the MiR05-Kit.

The preparation of the MiR05 medium described in this document may be performed only by personnel qualified for the specific task, such as technicians, scientists or trained students.

3. Preparation of a final volume of 250 mL MiR05

- Transfer the MiR05-Kit powder of one vial into a glass beaker.
- Add 230 mL H_2O.
- Dissolve with magnetic stirring at 30 °C.
- Add 3.75 mL of 5 M KOH at 30 °C and stir for 90 min.
- Adjust to pH 7.1 with 5 M KOH at 30 °C using a pH electrode. Do not use pH paper. The pH adjustment may be slow (90 min). pH has to be stable for at least 5 min. Do not leave the pH electrode in the solution during the 90 min waiting time.
- Separate approx. 50 mL of this prepared solution into a glass beaker and dissolve 0.25 g BSA (essentially fatty acid free) completely.
- Add the dissolved BSA to the main solution. Check pH again and re-adjust to 7.1 at 30 °C if necessary.
- Add H_2O to a final volume of 250 mL to complete the preparation of the medium.
- Partition the MiR05 medium in appropriate aliquots in plastic vials (e.g. 40 mL each) and store at -20 °C.

4. Storage information

- Aliquoted portions of prepared MiR05 medium can be stored at -20 °C for several months.
- Updated information on storage limitations will be provided on the website according to quality control in the OroborosO2k-Laboratory.
- The MiR05-Kit powder may contain gray or black specks.

- There could be a slight yellow coloring of the MiR05 medium: tests with isolated mitochondria (mouse brain) and cryopreserved HEK cells revealed no impact of the yellow coloring on respiration.
- Quality control: Every lot of MiR05-Kit is tested with HEK cells and compared to previous lots before release to the market.
- The stability over time of MiR05-Kit has been tested for lots #0915, #18.02872, #19.01689. The quality of MiR05-Kit is assured for a 3-year period.
- Quality control data will be available open access soon.

5. References and ordering information

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:431-42. - »Bioblast link«

Further information: » http://wiki.oroboros.at/index.php/MiR05-Kit

6. Author contributions and acknowledgements

Gnaiger E was responsible for the concept of the project. Gnaiger E, Cecatto C and Cardoso LHD prepared the MiPNet and all coauthors contributed to the final version. Immanuel Plangger and Miriam Hunger, as former members of the Oroboros Instruments, contributed to the project and to this MiPNet.

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