

Open peer review and authors' responses

Stability of mitochondrial respiration medium used in high-resolution respirometry with living and permeabilized cells

Authors: Eleonora Baglivo, Luiza HD Cardoso, Cristiane Cecatto, Erich Gnaiger

Bioenerg Commun 2024.8. <https://doi.org/10.26124/bec.2024-0008>

Reviewer 1: Jason N Bazil

Michigan State University, USA

Manuscript reviewed 2024-11-04: *Only major points included.*

Reviewer 1

The manuscript titled, "Stability of mitochondrial respiration medium used in high-resolution respirometry with living and permeabilized cells" by Baglivo et al. is a very well-written report that provides useful information about the long-term stability of the MiR05-Kit using HEK 293T cells. This information will be reassuring to other mitochondrial and bioenergetic researchers. The figures are of good quality and the level of detail is sufficient for others to reproduce the study results, if desired. The discussion is well-thought out and would explain why any future replication attempts may produce somewhat different quantitative results. I only have very minor suggestions and questions

Line 49: suggest adding the importance of water purity when making buffers. Water should be 18.2 MOhm purity to maximize the frequency of obtaining consistent results.

Authors

Information about water resistivity was added in section "2.2. Chemicals" (line 92).

Reviewer 1

Line 365: Could it also be that different passage numbers prior to cryo-preservation introduce a metabolic phenotype drift? I did not find any information on the number of cell passages the HEK 293T cells underwent. This idea is related to the information contained in the paragraph between lines 466 and 476 where the authors correctly point out the importance of knowing and understanding cell batch variations.

Authors

The range of cell passages was added in section "2.3 Sample preparation" (line 106). Since measurements in the same years used vials obtained from the same cell culture used for cryopreservation, the number of cell passages cannot influence the comparison between the lots of MiR05-Kit within any year (clarified in line 352). The possible effect of passage number on respiration in different years was added to the discussion (line 490).