**MS ID:  JoVE59052**

**MS TITLE:  Analyzing Oxygen Consumption Rate in Primary Cultured Mouse Neonatal Cardiomyocytes Using an Extracellular Flux Analyzer**

We would like to thank editors’ thoughtful comments. We have carefully considered all editorial comments, performed some additional analysis address some of the concerns, and revised the manuscript to address the issues that were raised. Please find below a summary of the modifications to the manuscript and a detailed point-by-point response to the editorial comments.

**Summary of modifications: (added figures and tables)**

1) Added new Table 1, showing how to make reagents and buffers, which are used in the protocol. This information is useful for readers to reproduce the procedure and experiments.

2) Added Figure 2C, presenting positive immunostaining of cardiomyocyte specific maker sarcomeric -Actinin in isolated neonatal cardiomyocytes. (to respond Reviewer’s concern)

3) The protocol is highlighted for identifying the essential steps of the protocol for the video.

**Point-by-point response to the editorial comments (editorial comments are in italics):**

**Editorial comments:**  
Changes to be made by the author(s) regarding the manuscript:

*1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.*  
A. We have proofread and corrected all spelling and grammar issues.

*2. Please revise lines 88-90, 170-171, 248-249 (Table 2), 282-284 to avoid previously published text.*A. The manuscript has been revised to avoid previously published text in the above mentioned lines. The new lines of the revised manuscript are lines 97-98, 181-182, Table 3, and 299-300.

*3. Please provide an email address for each author.*  
A. We provided email addresses in our manuscript. Lines 21-26.

*4. Please replace “XFe96”, which is commercial language, with another keyword.*A. We have replaced “XFe96” with “96-well format extracellular flux analyzer”.

*5. Please expand the Summary to briefly describe the applications of this protocol.*

A. The manuscript has been revised as suggested. Please see lines 428-436.

*6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by “(**see table of materials)” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: XFe96, Eppendorf, Wave, etc.*A. We have corrected all commercial language accordingly.

*7. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.*A. The protocol has been revised as suggested.

*8. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).*A. The protocol has been revised as suggested.

*9. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.*A. The protocol has been revised as suggested.

*10. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below.*A. The protocol has been revised as suggested. Please see the text.

*11. Lines 122-128: Listing an approximate volume to prepare would be helpful.*A. An approximate volume is added to the protocol.

*12. Line 140: Are the mice anesthetized before this procedure? If so, please mention how they are anesthetized and how proper anesthetization is confirmed.*A. The mice are not anesthetized. According to NIH and American Veterinary Medical Association guidelines, decapitation alone for neonatal mouse pups < 7 days of age, is an acceptable means of euthanasia.

*13. Line 148: What is used to cut?*A. The tool has been added to the text.

*14. Line 154: What is the concentration of trypsin?*A. The concentration has been added.

*15. Line 158: Please list an approximate volume to prepare.*  
A. A volume has been added to the text.

*16. Line 256: Please provide composition of RIPA cell lysis solution. If it is purchased, please cite the Table of Materials.*A. The source of RIPA solution has been added to the text.

*17. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.*A. The protocol has been revised as suggested.

*18. Please include single-line spaces between all paragraphs, headings, steps, etc.*A. The manuscript has been revised as suggested.

*19. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.*A. The essential steps in the protocol are highlighted.

*20. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Please do not highlight any steps describing anesthetization and euthanasia.*A. The protocol has been highlighted as suggested.

*21. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.*A. The details have been provided as suggested.

*22. Figures 2 and 4: Please change “hrs” to “h” for time unit.*A. The manuscript has been revised as suggested.

*23. Figure 3: Please define RAA in the figure legend.*A. The manuscript is revised.

*24. Table 1: Please change the unit “μl” to “μL”.*A. The manuscript has been revised as suggested.

*25. Please remove the embedded table(s) from the manuscript. Each table must be accompanied by a title/description after the Representative Results of the manuscript text.*A. The manuscript has been revised as suggested.

*26. Discussion: Please discuss any limitations of the technique.*A. The manuscript has been revised as suggested. Please see lines 437-443.

*27. References: Please do not abbreviate journal titles.*

A. The references have been revised as suggested.