Rebuttal document

Laser microdissection-based protocol for the LC-MS/MS analysis of the proteomic profile of neuromelanin granules

Dear Editor,

We want to thank you and the reviewers for the comments and recommendations concerning our manuscript “Laser microdissection-based protocol for the LC-MS/MS analysis of the proteomic profile of neuromelanin granules”. You can find responses to each of your comments down below. With this, we hope that the resubmitted version is acceptable for publication now.

Kind regards,

Maximilian Wulf, on behalf of all Co-Authors

Editorial comments:

Editorial Changes

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

The manuscript was proofread thoroughly for spelling and grammar issues.

2. Please revise the following lines to avoid previously published work: 108, 111-117, 123, 149, 202-206, 216, 226, 261.

All lines were revised accordingly.

3. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).

All speeds were converted with one exception (p.7 line 208), as no transformation into g is possible for the used thermomixer.

4. 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.
For example ThermoFisher Scientific, etc.

We removed commercial language in the context of software and instrumentation names.

5. Please add more details to your protocol steps. 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.
Step 1.2: Where was the stainless-steel knife installed?

For clarification, we revised the phrase “Clean the stainless steel knife with 70% ethanol and install it into the blade holder.” (line 109)

Step 1.5: What is section medium? Please define its composition.

As we used a commercial frozen section medium, we can unfortunately not define its composition.

Step 1.7: What is the optimum orientation for the tissue holder?

We included the following sentence in the manuscript “Optimal orientation depends on the orientation of your tissue.” (line 119)

Steps 1.8 and 1.10 are the same. Were cutting settings adjusted twice? If yes, what were the settings in step 1.8?

We removed step 1.8 from the protocol, as an adjustment of the cutting settings is necessary only before sectioning of the area of interest.

Step 1.14: The anti-roll plate contained the membrane slide? If yes, please mention this.

No, the anti-roll plate does not contain the membrane slides. For clarification, we added a more detailed description in lines 128 and 129 “Cut a section of the tissue, open the Anti-roll Plate carefully, take a membrane slide and prevent tissue folding while placing the tissue section on the membrane slide.”

Step 2.16: Please provide sonication settings used.

We added the used sonification settings (45kHz; line 198).

Step 3.7: How much trypsin was added?

We added 0.1 µg of trypsin per 1,000,000 µm² of tissue as stated in the NOTE belonging to former step 3.7 (now 3.6) “NOTE: For 1,000,000 µm², 0.1 µg of trypsin was found to be sufficient.”

Step 7.1: Please mention the proteins used in this study.

We addressed that comment by mentioning the protein cytoplasmic dynein 1 heavy chain 1, which was used in this study for PRM experiments “For our representative results, we selected cytoplasmic dynein 1 heavy chain 1, …” (line 308-309).

6. Please ensure that the table of materials contains all the reagents, equipment, glassware, etc. used in the study along with catalog number and company name.

From personal correspondence with one of the JoVE editors (Neethu Abraham), we got the information to only include those materials that were essential for the presented protocol. The included materials are essential, as used centrifuges, pipettes, mass spectrometer etc. could be exchanged.

7. Please include one line space between all step, substeps and notes in the protocol.

Done.

8. Please also include the following in the Discussion along with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique

We included all of these aspects in the last paragraph of the discussion (lines 434-455).

9. Figure 5: Please provide retention time unit.

The retention time unit is now provided in the labeling of the axis in figure 5.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

In this methodological protocol, Wulf et al. describe the complete workflow to characterise the molecular composition (proteins) of neuromelanin granules. The protocol is well-written covering from all experimental designs/settings to validation assays, including all parameters applied with all the softwares used. The detailed description of the manuscript makes it a robust protocol to be used not only by researchers with interest in neurological disorders but also by the scientific community with interest in the isolation and characterization of specific cells or aggregates in multiple biomedical contexts.

We thank Reviewer #1 for his comments to the presented protocol.

Reviewer #2:

Manuscript Summary:

very fine ms and protocol

We thank Reviewer #2 for his comments to the presented protocol.