• Please submit your manuscript as a Word document written in 12 pt font and single-spaced text.

**The manuscript has been adjusted to a 12 pt font and is single-spaced.**   
  
• Please scan and upload the Author License Agreement

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• JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. Please remove all commercial sounding language from your manuscript (text and figures). Examples of commercial sounding language in your manuscript are: Tandem Mass Tag, TMT, Jackson Laboratory, FastPrep 24, MP Biomedicals, Eppendorf, Pierce, Pierce Thermo Scientific, Protea Biosciences, SpinTips, Eksigent Nano LC, Orbitrap Elite, Fusion, Proteome Discoverer, Excel, etc. All commercial products should be sufficiently referenced in the table of materials/reagents. Please replace all commercial sounding language in your manuscript with generic names that are not company-specific.

**We have removed all commercial sounding language from the introduction, protocol (see steps 1.1.1, 1.2, 5, 6, 7.6 and 8.3.1) , results, discussion, and figure 1.**  
• Please ensure that all items mentioned have been included in the Materials/Equipment list, and are accompanied by a catalog number. For e.g., FastPrep 24 system, etc.

**All items mentioned in the manuscript are in the Materials/Equipment list and are accompanied by a catalog number.**  
• Please define all abbreviation before use. For e.g. ACN, etc.

**We have defined abbreviations at the first use within the text. See steps 1.2, 3 and 6.1.1 in the protocol.**   
  
• Please describe centrifuge speeds as "x g" instead of the machine-dependent "rpm".

**We have changed the centrifuge speeds from rpm to g. See steps 1.1.2, 3.3, 6.1.5, and 6.1.8 in the protocol.**   
  
• Please re-word the Short Abstract (10-50 words) to more clearly state the goal of the protocol. For example, “This protocol/manuscript describes…”

**We have re-worded the short abstract to clearly state the goal of the protocol. Please see the revised short abstract**:

***“Combined precursor isotopic labeling and isobaric tagging (cPILOT) is a quantitative proteomics strategy that enhances sample multiplexing capabilities of isobaric tags. This protocol describes the application of cPILOT to tissues from an Alzheimer’s disease mouse model and wild-type controls.”***   
  
• Introduction 2nd paragraph- typo- should be “combined” precursor...cPILOT).

**This typo has been fixed. Please see the second paragraph in the introduction.**  
• Please ensure that all text in the protocol section is written in the imperative tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.). 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”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

**The protocol section has been adjusted to reflect the imperative tense. Instances in which the imperative tense cannot be use have been made notes. Please see steps 7.4, 7.5, and 7.6 in the protocol.**  
  
• Step 2.1: In what volume?

**We have clarified this by adding information in step 2.1 that references step 1.2. The added volume is dependent on the protein concentration.**   
  
• Step 3.1: How are the samples thawed?

**We have updated the text in step 3.1 to describe how to thaw the samples.**

• 6.2: Please provide the centrifugal evaporation details.

**We have added the centrifugal evaporation details by referencing step 3.3 in the protocol.**  
  
• 7.4, and 7.5: Please re-write these steps in the imperative tense or add them as a Note.

**These steps have been re-written in the imperative tense. Please see steps 7.4 and 7.5.**

• 7.7 should be a Note. Ran should be run.

**We have changed step 7.7 to a note and instance of “ran” have been changed to “run”.**   
  
• Section 8: Please provide a reference for data analysis.

**We have added a reference for the data analysis. The reference is below.**

Evans, A. R., Gu, L., Guerrero, R. & Robinson, R. A. S. Global cPILOT analysis of the APP/PS-1 mouse liver

proteome. *PROTEOMICS – Clin Appl.* 9 (9-10), 872-884, doi:10.1002/prca.201400149, (2015).

• After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight (in yellow) 2.75 pages or less of text (which includes headings and spaces) to identify which steps should be visualized to tell the most cohesive story of your protocol steps. Please see JoVE’s instructions for authors for more clarification. Remember that the non-highlighted protocol steps will remain in the manuscript and therefore will still be available to the reader.

**We have ensured that the protocol is within the 10-page limit. Please include all sections of the protocol except Sections 6.1, 6.2, and 8.3 for filming.**

• Please disregard the comment below if all of your figures are original. If you are re-using figures from a previous publication, please obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]." We do not require the permission to re-use the figures until after internal and peer review. You can however, upload the permission to re-use email/file any time during the review process via your editorial manager account.

**This comment is not applicable as both of the figures are original.**

• Please ensure that your discussion covers the following in detail and in paragraph form:

1) modifications and troubleshooting,

**This part of the discussion has been addressed and includes a discussion of how cPILOT can be modified with other isobaric tags and issues that may arise if the incorrect pH is used. Please see the first paragraph of the discussion section.**

2) limitations of the technique,

**This has been addressed in the discussion section. Please see the second paragraph of the discussion section.**

3) significance with respect to existing methods,

**We have added a sentence to the discussion section below.**

***“This method, similarly to other multiplexing methods, allows for the analysis of multiple samples at once, and is applicable to samples originating from cells, tissues, bodily fluids, or whole organisms.”***

4) future applications and

**We have added the sentence below.**

**“In the future, cPILOT will be expanded to multiplex a larger number of samples by using existing and novel isotopic and isobaric labels.”**

5) critical steps within the protocol.

**This part of the discussion has been addressed in the discussion section, whereby we highlight importance of using the correct pH, performing dimethylation prior to isobaric tagging, and give commentary on how to handle the samples. Please see paragraph one of this section.**

• References: Please abbreviate all journal titles.  
**We have abbreviated all the journal titles. Please see the reference section.**