Editorial comments:

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 JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

A : We revised carefully.

2. Please shorten the title. Suggested: studying protein import into chloroplasts using protoplasts

A : We changed the title as you suggested

3. Figure 1: Please label the panels. Please use larger figures with higher resolution.

A : We labeled panels and saved figures of the original size for higher resolution.

4. Figure 2: Please use SI abbreviations for time: h instead of hr.

A : Corrected.

5. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.

A : Corrected.

6. Please include a Summary that clearly describes the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

A : We added a Summary

7. 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.

A : We described protocol steps in more detail.

8. What is an e-tube?

A : It is an abbreviation of the Effendorf-tube. We described it in the manuscript.

9. 2.1: How much is used to dissolve?

A : 100 µl of distilled water

10. 3.3: Centrifuge for how long?

A : It’s 10 min.

11. Please specify all centrifugation time lengths.

A : We specified time lengths.

12. 4.3: What is the proper amount?

A : It’s 10 µg

13. 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. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

A : We highlighted steps

14. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. 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.

A : We organized highlighted steps according to the logical flow.

15. Please do not abbreviate journal titles.

A : Corrected