

Responses to the Second Editorial Comments

Responses to Editorial Comments

We thank the editor for the detailed comments and questions regarding the protocol. We have addressed all comments and have included detailed responses below as well as in the manuscript.

Comment 1:

We appreciate the edits to the highlight. After a detailed review, the interfacing step appears to be critical to the success of the technique. Hence, we included section 6 in the highlight and adjusted the highlight a bit elsewhere to ensure length limits. After editing your steps as requested, please ensure that the total highlight does not exceed 2.75 pages.

Including a spacing between the steps, the total length currently exceeds 2.75 (it is close to 3 pages). Editors can decide which step to cut further to bring the total length under 2.75 pages.

Comment 2:

We added this here. Please ensure that Figures are listed in the order that they appear.

We spread out the figures in the protocol section a little bit. We also re-numbered some of the figures (Figures 3-6) to ensure they appear in the revised order.

Comment 3:

Please mention the chosen euthanasia method in a note.

We purposefully did not elaborate on this point since different institutions have different protocols regarding proper euthanasia. We've included the suggested information in this revision however.

Comment 4:

When is the mesh used? What kind of mesh is used? Please provide specifications.

The mesh was a late addition in response to the last review. Our apologies for not including more details here. It was included in the Table of Materials and Equipment however.

Comment 5:

How? Mention button clicks/menu selections.

Revised the sentence mentioning clicks.

Comment 6:

What is the rate of perfusion?

Perfusion rate has been added.

Comment 7:

Mention magnification and lens N.A.

Magnification and N.A. detail has been added.

Comment 8:

How is this done? Is this a manual knob turn on the pressure injection device?

We had previously neglected to mention that the suction pressure for the bottom perfusion was maintained by a separate system with a manual control valve. Now, we have included this device (vacuum waste kit) in the revised Table of Materials and Equipment as well.

Comment 9:

Is there an image to show how this would look?

We unfortunately do not have any image available to show how this would look. Since this is a dynamic process, we believe it would be best displayed through a video rather than an image.

Comment 10:

How do you do this?

We intended this to be a segue from the previous point, not a process in and of itself. We have altered the wording to more correctly describe the procedure

Comment 11:

What mesh is this? This is the first time it is mentioned in the protocol

This is the same mesh used in step 3.6. Please see our reply to the previous comment regarding the mesh. Hopefully the additional details added in response to that comment will also answer this one.

Comment 12:

Do you use forceps?

Yes we do use forceps to peel the mesh from the retina and have indicated this in the revised text.

Comment 13:

We added this here. Please ensure that Figures are listed in the order that they appear.

This is okay

Comment 14:

Manually?

Yes, manually. Sentence has been revised accordingly.

Comment 15:

How is this done? If we are to film this, all actions must be described.

The necessary detail has been added.

Comment 16:

What is the glutamate concentration?

The glutamate concentration (1 mM) was already mentioned above in step 5.1, so we didn't want to repeat here. But we have revised the sentence to include this detail again.

Comment 17:

What is the glutamate concentration?

The glutamate concentration (1 mM) was already mentioned above in step 5.1, so we didn't want to repeat here. But we have revised the sentence to include this detail again.

Comment 18:

How is this done?

By pressing the 'Fill' button –the sentence has been modified to reflect this.

Comment 19:

Please mention button clicks and software menu selections.

This is now mentioned.

Comment 20:

Please mention button clicks and software menu selections.

This is now mentioned.

Comment 21:

Please mention button clicks and software menu selections.

This is now mentioned.

Comment 22:

Please mention button clicks and software menu selections.

This is now mentioned.

Comment 23:

Mention pressure in each injection and durations.

Pressure and durations of injections have been added.

Comment 24:

For ALL steps here, Please mention button clicks and software menu selections to describe the actions. We cannot film steps with general descriptions.

Button clicks and menu selections have been added for all steps.

Comment 25:

If the green LED is not use, what is turned ON and OFF?

We mean that the same exact procedure was followed as in 7.2.1 except the LED was not turned ON (i.e. ON and OFF were exactly the same). We have tried to simplify this in the revised text

Comment 26:

Unclear what you do here. What do you mean by a trial here?

We have simplified this in the revised text

Comment 27:

Please add scale bar.

Scale-bar has been added to this fire. Note that this figure (old Figure.6) is now renumbered as Figure. 3 after reordering the figures.

Comment 28:

Please add scale bars on all panels.

Scale bars have been added to all panels of this figure. Note that this figure (old Figure 5) has been renumbered as Figure 4 after reordering the figures.