Vineeta Bajaj

JoVE  
1 Alewife Center

Suite 200

Cambridge, MA 02140

San Diego, August 28th 2018

Dear Dr. Bajaj,

Thank you again for your consideration of the submitted manuscript “Optimized Interferon-gamma ELISPot assay to measure T cell responses in the guinea pig model after vaccination” for publication in JoVE. Please extend our gratitude to the editors and reviewers for providing very valuable feedback and suggestions regarding our work. We will respond to each of the comments specifically in a point-by-point manner. It was very encouraging to read that our peers see high value for this assay for immunology studies in guinea pigs.

**Addressing editorial comments:**

*“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.*

Author Reply: Thank you for the opportunity. We have made minor corrections throughout the manuscript. (lines 69/70, 101, 120, 123, 373, 397, 475, 503)

*2. Figure 3B: Please delete the ^ symbol and use superscript for the exponent (i.e., 6).*

Author Reply: Figure 3B is updated.

*3. Please provide an email address for each author.*

Author Reply: *E*mail-addresses for each author have been added on page 1 of the revised manuscript.

*4. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.*

Author Reply: SI abbreviations are now used throughout the manuscript (lines 160, 182, 183, 203, 209, 210, 223, 226, 230, 233, 235, 239, 246, 250, 255, 259, 266-267, 278, 281, 284, 292, 302, 306, 313, 316, 324, 329, 259, 366, 393)

*5. Please convert centrifuge speeds to centrifugal force (x g) instead of rcf.*

Author Reply: All mentioned centrifuge speeds have been converted from rcf to x g unit (lines 208, 210-211, 228)

*6. Please delete the ^ symbol and use superscript for the exponent (i.e., 6).*

Author Reply: All exponents, including figures, are now in superscript (lines 394, 367, 375, 376,).

*7. 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. For example: CELLECTRA®, Ficoll-Paque Plus, SepMate™, STEMCELL Technologies, etc.*

Author reply: Throughout the manuscript trademark symbols, brand and company names have been removed (lines 168, 201-206, 208-211, 220, 352, 354-355, 357, 360, 366, 384, 391-394, 399-400, 428, 431-436). Commercial products are referenced in the revised Table for Materials and Reagents.

*8. Please revise the protocol to contain only action items that direct the reader to do something. 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 move the discussion about the protocol to the Discussion.*

Author reply: The protocol has been revised to include only action items (lines 143, 158, 163, 194, 253).

*9. 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.*

Author reply: More details have been added to the protocol steps (lines 169, 176-177, 185, 188, 199, 201, 203, 215-216, 218, 220-223, 228-231, 255-257, 264, 269, 273, 297-298, 300, 308-311, 318-327, 335-337) . In addition, literature references to provide the reader with further details regarding animal anesthesia (lines 148-149, 170-172, 179/180), Mantoux injection technique (lines 164/165), non-terminal bleeding (183) and Trypan-Blue staining (line 237) have been added.

*10. 1.1.1: Please specify the gender and age of guinea pig. Please mention how proper anesthetization is confirmed.*

Author Reply:Guinea pig age and gender have been specified (line 145). We added new sub-step 1.1.2 (line 148/149) to provide instructions detailing anesthesia. We also expanded 1.2.4 (170-172) to specify how to monitor animal’s recovery. We provided a reference regarding monitoring of anesthesia in the discussion part (lines 416-420).

*11. 1.2.1: Please describe Mantoux technique or add a reference.*

Author Reply: We have provided a reference describing the Mantoux skin injection technique.

*12. 3.1.3: What happens after the centrifugation? Is supernatant discarded? Please specify throughout.*

Author Reply: The handling of tubes after each centrifugation step in the protocol is detailed, we added new sub-step (3.1.4) to clarify to not pour off any content after density centrifugation.

*13. 3.1.6: What volume of R10 medium is used to wash?*

Author Reply: The volume of R10 medium to use for washes has been specified *in substep 3.2.7 (line 230-231).*

*14. 3.1.8: Please describe how this is actually done.*

Author Reply: We added a literature reference to provide details about the Trypan-Blue stain (line 237).

*15. 4.1.1, 4.1.2: Please describe how to pre-treat plates with ethanol. What is the incubation temperature?*

Author Reply: The description of ethanol-pretreatment of plates has been provided and incubation temperature specified (lines 246-251).

*16. 4.2.2: This step does not have enough detail to replicate as currently written. Alternatively, add references to published material specifying how to perform the protocol action.*

Author Reply: We modified sub-steps in section 4.2 to clarify what is required for cell plating and to contain only action items (lines 273-288).

*17. In the JoVE Protocol format, “Notes” should be concise and used sparingly. They should only be used to provide extraneous details, optional steps, or recommendations that are not critical to a step. Any text that provides details about how to perform a particular step should either be included in the step itself or added as a sub-step. Please consider moving some of the notes about the protocol to the discussion section.*

Author Reply:Thank you for the clarification on the use of “notes”. We have converted most of them into protocol steps (lines 185, 196-197, 246-248, 250-251, 314).

*18. Please include single-line spaces between all paragraphs, headings, steps, etc.*

Author Reply:Spacing is now set to ‘single’ for the entire document.

*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.*

Author Reply: We have highlighted the sections of the protocol that should be video-taped. The video should follow the sequence of steps, e.g. section 4.1.1 through 4.1.7 before section 3.

*20. 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.*

Author Reply: All relevant details required to perform the step in the highlighting have been provided.

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

Author Reply: Reference list now displays full journal titles.

*22. Table of Equipment and Materials: Please provide lot numbers and RRIDs of antibodies, if available.”*

Author Reply: Table of Equipment and Materials is updated and contains all available information.

**Addressing reviewer 1, major concerns**

*“There is one key aspect which undermines the usefulness of publication of this protocol and that is the source of the antibodies. It states that these were generated (without providing the methods) from B cell clones provided by one of the co-authors. For this assay to have any use to the scientific community, there needs to be a commercially available source of the antibody pairs. Without this, each laboratory will need to generate their own reagents in a non-harmonised manner leading to lab-to-lab variation in the assay. Furthermore, there will be labs who would wish to use the assay but cannot because they lack the facilities / resources for antibody production/purification etc. The authors need to comment on this aspect because the assay is completely dependent on the quality of these critical reagents.”*

Author Reply: The original clones were developed by Dr Hubert Schaefer, co-author of this manuscript, and his lab. They have addressed the issue of commercialization. Dr Schaefer has an arrangement with the German company ImmunoTools that ensures the availability of the antibodies to the scientific community. Researchers should first place requests to Dr Schaefer’s lab, before being referred to ImmunoTools. Production, delivery and invoicing is completely handled by ImmunoTools. The antibodies are available in different formats (biotinylated, HRP-conjugated, fluorochrome-conjugated). Researchers interested in using this assay therefor do not need to have the facilities or resources to produce and purify antibodies. Although a new batch will be manufactured for each placed order, the manufacturing process takes place in a regulated commercial setting that results in consistent product characteristics.

We have summarized this procedure in the discussion section (460-463), so readers are informed about how to order the antibodies. Just to clarify: The antibodies used in our lab to generate the data presented in this manuscript were manufactured by GenScript from the same cell-line.

**Addressing reviewer 1, minor concerns**

*Line 145: do you mean 'equal' or should it be 'optimal'*

Author Reply: Line 145 (line 208-211 in revised manuscript) has been removed because it was not an action item.

*Line 153: the term 'Trypan Blue exclusion test' would be better here*

Author Reply: Line 153 (line 237 in revised manuscript) We agree with the reviewer and specified the test-name accordingly throughout the manuscript (lines 237, 395, 440).

*Line 187: 'empty' implies removal of all of the contents whereas is only supernatant that is being removed*

Author Reply: Line 187 Changed wording throughout the protocol to clarify, that only supernatant/buffer is removed from wells.

*Lines 190 and 194: it is surely a 0.22um filter, not 22um.*

Author Reply:Lines 190 and 194 (lines 303 and 314 in revised manuscript). The Reviewer is correct, wording changed to clarify the size of filter to be used for this step.

*Lines 200 to 202. Although there is merit in highlighting a particularly toxic reagent, this is not the only chemical in the protocol for which caution should be exercised and there should be a general point about referring to MSDS's and performing risk assessments.*

Author Reply: Lines 200 to 202 (329 to 331 in revised manuscript): We added a general comment regarding MSDSs and PPE at the beginning of the protocol section (line 137-138).

*Line 206: perhaps a recommendation could be given to use a plate reader since this is preferable for storage of the data, more objective analysis etc than manual counting.”*

Author Reply: Line 206 (344 of revised manuscript). This is a very valid point and we added a recommendation for an automated system in the discussion part (lines 448-451). We left the decision regarding the counting method in the protocol section more open to the discretion of the protocol-user, since the automated ELISpot plate reader is expensive and not standard lab equipment. The absence of it should not prevent researchers from using the described assay.

**Addressing reviewer 2, minor concern**

*“This reviewer believes the authors should state what vaccine was being tested. Some comments suggest it may have been an influenza vaccine. T cell responses differ upon intradermal versus IM delivery of plasmid. A brief discussion of the vaccine being tested is warranted.”*

Author Reply: We include a paragraph (473, 482-490, 500) in the discussion section that lists previous work with the same pDNA vaccine used in this manuscript. This literature overview encompasses various species (mice, guinea pig, rabbit) and delivery routes (intra-dermal, mucosal, intra-muscular), in all of them the used pNP vaccine generated robust immune responses in combination with EP. We also made minor additions to introduction (line 69/70) and representative results section (line 391/392) to emphasize that we worked with an Influenza vaccine to generate data for this manuscript.

Again, we thank the reviewers and journal editors for the excellent critique and promptness. We hope we have answered your concerns and applied your suggestions sufficiently to bring this manuscript up to a level ready for video production by JoVE.

Sincerely,

Katherine Schultheis and Trevor Smith

Katherine Schultheis

Scientist

Inovio Pharmaceuticals, Inc.

10480 Wateridge Circle

San Diego, CA 92121

Trevor Smith

Associate Director

Inovio Pharmaceuticals, Inc.

10480 Wateridge Circle

San Diego, CA 92121