

Thank you for preparing a manuscript for the *Journal of Visualized Experiments*.

JoVE scope and general submission guidelines

JoVE is a peer-reviewed publication comprised of proven and novel techniques. The journal is based on publishing written protocols accompanied by a video demonstration of the technique, procedure or method. The scope of *JoVE* is broad; we publish protocols for methods which are better explained visually. We publish topics ranging from the most basic biology protocol to the most complex medical procedure. *JoVE* publishes in the areas of biology, medicine, chemistry, engineering and physics. At this time, *JoVE* only publishes protocols and methods with representative results. *JoVE* is not a results based journal.

Generally, the authors of the protocol must have previously used their method to produce results reported in a peer-reviewed, results-based journal. Nevertheless, we appreciate that protocols are constantly evolving. Thus, if the methodology has been improved upon since the original publication, we invite our authors to publish the modifications that they have made and provide us with the latest version of the protocol, as this will be most useful to our readers.

Unless we are notified otherwise in a cover letter on submission, submission to *JoVE* is taken to imply that there is no significant overlap between the submitted protocol and any other protocols from the same authors under consideration or in press elsewhere. In cases where authors have previously published their protocols, please ensure that there is no direct repetition of text between this protocol and previous publications of the method, as this would constitute self-plagiarism.

Submission is also taken to imply that all coauthors have approved the contents of the protocol and its submission by the corresponding author, and that the corresponding author is authorized to represent all coauthors in pre-publication discussions with the journal. (The corresponding author for editorial purposes need not be the senior author, not the person to whom correspondence is addressed after publication.) The primary affiliation for each author should be the institution where the majority of their work was done. If an author has subsequently moved, the current address may also be stated.

All articles will undergo peer review. Some authors are commissioned to write an article; this does not guarantee publication. In addition, the journal reserves the right to retract a protocol, even after it has been accepted, if it becomes apparent that there are serious problems with the scientific content or with violations of our publishing policies.

Manuscript tracking system

Please use our [manuscript tracking system](#) to submit your protocol. If you have been invited to submit a protocol, specific links for submitting your manuscript are supplied in e-mails from the editors.

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Production options

JoVE offers professional video production services. We understand that most researchers and clinicians do not have video production experience. Therefore, we handle all pre- and post-production work for you. This includes script and storyboard writing, trained videographer services, professional video editing services (including graphic artists, voice-over talent, and science editorial review). The author provides the written manuscript, which is then scientifically reviewed. Once accepted, our qualified JoVE Production Services staff create the video.

Authors are welcome to produce their own videos. However, the videos must meet the same rigorous publication standards of a JoVE produced video. They must fit within the JoVE format and style as well as meet the technical guidelines specified on www.jove.com/publish/author-produced. Author produced videos receive no editing assistance from the JoVE production staff. Author produced videos and manuscripts are editorially and peer-reviewed simultaneously, and therefore must be submitted together. Further details of the author produced video submission policies can be downloaded [here](#). **NOTE:** *This publication option should only be selected if the authors have the technical and professional expertise to edit video and audio.*

Timeline for JoVE produced videos

After submission of your protocol, it will be editorially and peer-reviewed. If the text article passes review, a script will be generated. Generally, a filming date will be scheduled for 4-8 weeks *after acceptance*. A videographer will be sent to your lab to film a demonstration of the procedure. You will need to have everything prepared so filming can take place within one day. Following filming, footage will be sent back to our video editing studios where it will be spliced together and professional voiceovers, animations and other text and graphics added. Once a rough cut is generated (typically 6-8 weeks post-filming) you will get to review the video to ensure it correctly represents your protocol. Any necessary scientific changes will be incorporated into the video. Following your final approval, your video and text article will be published on the JoVE website and indexed in PubMed/MEDLINE. In general, it takes 5-8 months from submission to publication. Please note that any protocol with animal work requires an additional veterinary review; these articles may take longer than normal to be published.

Timeline for author produced videos

After submission of your protocol and a low resolution version of your video (<50 mb), the manuscript and video will be reviewed by both our editorial and production staff. This review can take several weeks. Please, only select this publication option if you or colleagues have the technical expertise to edit video and audio; frequently, authors need to make multiple revisions to the video to meet our criteria. If the video meets our stringent [author produced video criteria](#), both the text and a full resolution version of the video will be sent out for peer review. If accepted, your video and text article will be published on the JoVE website and indexed in PubMed/MEDLINE. In general, it takes 4-7 months from submission to publication. Please note

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that any protocol with animal work requires an additional veterinary review; these articles may take longer than normal to be published.

Publishing options

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Before submitting, please ensure that you have the following sections in your manuscript (Please note, more detailed instructions are below):

- Title (maximum 150 characters)
- Authors (including affiliations, physical address and contact information)
- Corresponding author
- Keywords (4-10 words)
- Short Abstract (50 words maximum)
- Long Abstract (150-300 words)
- Introduction
- Protocol (2-3 pages, written in imperative tense, in step-by-step format)
- Representative Results (written in full sentences and paragraph form)
- Figures and Tables (require at least one figure or table)
- Discussion (written in full sentences and paragraph form)
- Acknowledgements (include acknowledgement of funding sources)
- Disclosure (conflict of interest statement)
- Table of specific reagents and equipment (use template)
- References (require at least 10)

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In your submission you will need to upload: a cover letter, list of 3-5 reviewers, manuscript, [table of materials/reagents](#), figures as separate files and the [author license agreement](#).

Detailed Instructions

Your manuscript must include the following information. Failure to follow these guidelines will delay the editorial and peer review process, placement on the videographer schedule, and ultimately publication turnaround time.

Required formatting

As we use Microsoft Word in the Editorial office, this is our preferred format for text. Please use 12 pt Arial, Calibri, or Times New Roman fonts throughout the document. Page margins must be 1 inch on each side. Line spacing should be 1 or 1.15. There should be a space between each paragraph/bullet point.

Manuscript Contents

Title: (maximum 150 characters) The title should succinctly describe the method and its application. For example: *DNA transfection of E.coli using heat*

Authors: Include those who have contributed intellectually and practically to the development of the technique. For all authors please include first name, middle initial (if appropriate), last name, postal address (also including Department, Institution, City and Country) and e-mail address. Please include the telephone number and fax number for the corresponding author.

Corresponding author: Include first name, middle initial (if appropriate), last name, postal address (Department, Institution, City and Country) and e-mail address. Also include the telephone number and fax number for the corresponding author.

Keywords: (minimum 4, maximum 10) Keywords should reveal the internal structure of the paper's reasoning. For example: *neuroscience, brain, mouse, transplantation, labeling*

Short Abstract: The short abstract should include a general description of the article and its applications. This description should focus around the procedure, not the results obtained by the procedure. (10 words minimum, 50 words maximum)

Long Abstract: The long abstract should include a more detailed description of the article and its applications. We would prefer that the abstract contain information about the procedure rather than a summary of the results that have been obtained previously using the method. (150 words minimum, 300 words maximum)

Introduction (use heading 'INTRODUCTION'): Include statements describing the significance of this method and rationale behind its implementation – particularly with respect to advantages over existing methods. The purpose is to enable readers to make a decision as to the suitability of the protocol to their experimental problem. Initially, you should introduce the technique under discussion. Include references to key papers where the protocol has been used

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previously, including those published by your own group or to reviews that discuss applications of the protocol (see below for how to cite references). Sufficient background information and a clear statement of the overall goal of the method are also required. You are actively encouraged, where appropriate, to reference other protocols in *JoVE*. (150 words minimum, 1500 words maximum)

Step by step methodology (use heading ‘PROCEDURE’): This is the major part of the protocol and must be a numbered list, ideally with numbers in **bold**. Please use active tense. For example: Pipette 20 ml of buffer A into the flask **Not:** 20 ml of buffer A are/were pipetted into the flask.

If the protocol naturally breaks into separate stages, include subheadings and resume the numbered list. Subheadings are particularly appropriate after steps in the protocol where the procedure can be stopped, i.e., when the experiment can be stopped and resumed at a later date. Any pause points should be indicated with a brief description of the options available. For example: Can be left overnight at 4 °C or frozen for up to a month at -20 °C.

A) Length – There is, technically, no limit to the amount of text you may write for the protocol section. However, if you are using *JoVE* Production Services, we can only film a limited amount of content for a single video article. To ensure that the filming of your video can be completed in a single day, we have a three-page limit on the length of the protocol text that is to be filmed. Please note: you do not need to delete any steps from your protocol. Should the protocol portion of your manuscript require more than the three-page text limit, authors are asked to identify (via highlighting with yellow) a total of three pages of protocol steps that are the most important to film. When highlighting, keep in mind which steps need to be visualized and which steps are sufficiently describe in text. The full length manuscript will be published along with the video; all unfilmed steps will still be available for people to read. We measure these three pages with the following formatting: 12 pt text, 1 inch margins, single-spaced, with a space between each step.

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B) Style – Your text manuscript should be a step-by-step description of the experimental procedure written in imperative tense. Write as if you were telling someone how to perform the technique. Emphasize critical steps in the protocol which must be performed in a very precise manner, providing the user with hints to increase the likelihood of success. Point out any toxic or harmful chemicals that are used. Make these clear by preceding them with the wording ‘CAUTION’ prior to their first mention and include brief details of the hazard and the appropriate handling information.

C) Format – Your protocol should be formatted to closely match the example below, with major sections being given a single digit number and individual steps within each section being given a two digit number. Each step should be no more than 2-3 sentences, not exceeding 6 lines of

text. A space should be inserted between each step of the protocol. Include figures with diagrams and/or photographs of equipment set-up, where appropriate. If the protocol is complicated, you should consider including a flow diagram to demonstrate how the stages fit together.

Example (protocol):

2) Stabilizing Peptides for Cross-Linking

2.1) Before solubilizing the HFIP-treated peptides for cross-linking reactions, one needs to prepare the cross-linking and quenching reagents.

2.2) Weigh out ammonium persulfate (APS, M_r 228.2 g/mol) and prepare a 20 mM solution in 10 mM sodium phosphate, pH 7.4. Mix using a vortex until the solution is clear.

2.3) Prepare 1 mM solution of Tris(2,2-bipyridyl)dichlororuthenium(II) hexahydrate (RuBpy, M_r 748.63 g/mol) in 10 mM sodium phosphate, pH 7.4. Mix using a vortex and verify complete dissolution. Protect the tube from light using aluminum foil.

2.4) For SDS-PAGE analysis following cross-linking, a convenient quenching reagent is 5% β -mercaptoethanol in 2 \times SDS-PAGE sample buffer. Alternatively, 1 M dithiothreitol (DTT, M_r 154.5 g/mol) in deionized water or a suitable buffer can be used.

2.5) HFIP-treated peptide films are dissolved in dilute NaOH first and then sodium phosphate buffer is added. Add 60 mM NaOH followed by deionized water into the tube containing the peptide film such that NaOH and water constitute 10 and 45% of the final volume, respectively. Scrape the peptide film off the inside walls of microfuge tube using the tip, mix by pipetting up and down, and sonicate for 5 min in a water-bath sonicator.

Representative Results (use heading 'REPRESENTATIVE RESULTS'):

Please add a concise, written description of a “representative” outcome following the use of this protocol, so that a viewer will have a sense of what a “good” or “bad” result looks like. Include information about, or examples of, the likely outcome. For example: likely yield of protein, typical microscopy images, etc.

If possible, please include data from an experiment that worked very well and a second for an experiment that required troubleshooting to obtain meaningful results. If not described in detail in the introduction, this is a good place to include directions on how to interpret and analyze the raw data, including any necessary equations. This is the appropriate section to include any analytical data for chemical compounds synthesized as part of the procedure. The representative results section should allude to any submitted figures in complete sentences and paragraph form. An example of how this should be formatted is shown below.

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Example:

The medium lifespan of *C.elegans* is temperature dependent. Figure 1B shows that temperature dependent changes in lifespan are accurately reproduced by the microtiter based lifespan assay²². Similarly, the microtiter plate assay reproduces changes in lifespan from mutants reported to have lifespans that differ from wild type N2 animals^{23-24 25} (Fig 1C).

The assay also can be used to make more quantitative statements. In Fig1D mean lifespan is plotted as a function of Mirtazepine concentration⁷. Each data point represents the mean lifespan of 7-12 population, each living in a different well. Even though the number of animals per well is relatively low (5-15 animals) the well-to-well variation is relatively small as can be seen from the error bars.

Tables and Figures (Required)

If using *JoVE* Production Services, please make sure that the text in all tables and figures is Arial font no less than 20 pt in size. Many, if not all, of your figures will be incorporated into the video presentation. For this reason, we ask that certain requirements on figures be met:

- Submit images separately as vectorized .ai, .eps, or .psd files. Layered .tiff files are also acceptable, though should be at least 720X480 pixels in dimension or 300 dpi.
- Figure panel labels and highlighting particular aspects of a figure (ex. Notable anatomical structure) should be on a distinct layer. Please note that we may ask you to submit each individual figure panel as unique file for incorporation into the video.
- Contain axis or axis tick labels greater 20 pt font.
- Contain lines with a weight no less than 3 pt.

All tables and figures should be given an appropriate title and should have a corresponding figure legend. We recommend at least one figure that graphically depicts the overall scheme of the experiment.

For authors creating their own videos, you will still need to upload figures as individual files in the same format as above.

Discussion (use heading 'DISCUSSION'):

Please remember that *JoVE* articles are focused on the method and protocol. Thus, the discussion should similarly focus on such. This section should include discussion on critical steps, limitations, possible modifications and trouble-shooting, future applications or directions after mastering this technique, and significance of the technique with respect to existing methods, etc. This should be written in full sentences and paragraph form. In general, this should be a minimum of 4-6 paragraphs of information.

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Acknowledgments (use heading 'ACKNOWLEDGMENTS'): List acknowledgements and all funding sources for this work.

Disclosures (use heading 'DISCLOSURES'): The published manuscript will indicate if the authors have competing financial interests. This statement will be published at the end of all papers, whether or not a competing financial interest is reported. Please include a version of one of these statements in your manuscript:

Examples:

- The authors declare that they have no competing financial interests.
- The author, [full name], is an employee of [full company name].
- The authors received funding that was provided by [full company name], which produces reagents and instruments used in this Article.

Table of specific reagents and equipment:

Include a list of the essential materials, split into reagents (use heading 'REAGENTS') and equipment (use heading 'EQUIPMENT') in table format. Please use the Excel [template](#) found on our website. These tables should include information about the suppliers used for reagents (e.g., company, web address and catalog number). Toxic or harmful agents should be made clear by the word 'CAUTION' followed by a brief explanation of the hazard and the precautions that should be taken when handling the agent. Please also highlight any specialist equipment required.

The information you provide about the sources of reagents and equipment will enable us to link your protocol to a product finder tool to help users obtain the equipment and materials they need. Please provide as much information as you can to help us successfully identify the product, i.e., company name, company website, product code. Please include this table within the text manuscript as well as in a separate Excel file.

Example (Reagent):

Name of the Reagent	Company	Catalogue Number	Comments (optional)
DMEM	Invitrogen	ABCD1234	

Example (Equipment):

Material Name	Company	Catalogue Number	Comments (optional)
Eye Tracker	SR Research Ltd.	Eyelink II	
Experimental Control Software	SR Research Ltd.	Experimental Builder	

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References (use heading 'REFERENCES')

List all references mentioned in the protocol. References are numbered sequentially as they appear in the text, figure legends, tables and boxes. Use superscript numbers to indicate a reference, for example ¹. Only one publication is given for each number, and footnotes may not be used. Patents should be included in the reference list. Only articles in formal, peer-reviewed online journals should be included in the reference list. Published conference abstracts and URLs for web sites should be cited parenthetically in the text, not in the reference list; grant details and acknowledgments are not permitted as numbered references.

For citations, all authors should be included in reference lists unless there are more than six, in which case only the first two authors should be given, followed by 'et al.'. Authors should be listed last name first, followed by a comma and initials of given names. Titles of cited articles are required and should be in Roman text and titles of books *in italics*; the first word of the title is capitalized, the title written exactly as it appears in the work cited, ending with a period. Journal names are *italicized* and abbreviated (with periods) according to common usage. Volume numbers appear in **bold**. For example: [Lastname, F. I., Lastname, F. I., et al. Article Title. *Source*. **Volume** (issue): FirstPage – LastPage, DOI, (YEAR).]. For book citations, the publisher and city of publication are required (e.g., John Wiley & Sons, Hoboken, New Jersey, USA, 2003).

Please provide **10 or more references** and list all references mentioned in the protocol. If you use the EndNote® reference management software, use the [JoVE output style](#) provided on the JoVE or EndNote websites. Please see the examples below:

1. Brenner, V., Lindauer, K. et al. Analysis of cellular adhesion by microarray expression profiling. *Journal of immunological methods* **250** (1-2), 15-28 (2001).
2. Klos, C. L., Shellito, P. C. et al. The effect of neoadjuvant chemoradiation therapy on the prognostic value of lymph nodes after rectal cancer surgery. *American journal of surgery* **200** (4), 440-445, doi:10.1016/j.amjsurg.2010.03.013 (2010).
3. Cho, J. K., Hong, K. Y., Park, J. W., Yang, H. K. & Song, S. C. Injectable delivery system of 2-methoxyestradiol for breast cancer therapy using biodegradable thermosensitive poly(organophosphazene) hydrogel. *Journal of drug targeting* **19** (4), 270-280, doi:10.3109/1061186X.2010.499461 (2011).

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In your submission, you will need:

- 1) Cover letter describing why your work should be published in *JoVE's* unique multimedia format
- 2) List of 3-5 peer reviewers names with their institutional affiliation and email address
- 3) Manuscript in the proper format
- 4) Separate figure files in the proper format
- 5) Separate Excel spreadsheet with [Table of Equipment and Reagents](#)
- 6) A signed [author license agreement](#). This can also be mailed or faxed to the editorial office.

Other Requirements

Authors are responsible for obtaining permissions for tables, figures, images or movies previously published elsewhere. If your tables, figures (altered or original), images or movies have been previously published, please cite their original publication within the legend.

For protocols that use live vertebrates or higher invertebrates, authors must state that all experiments should be performed in accordance with relevant guidelines, regulations and regulatory agencies. For manuscripts reporting experiments on human subjects, authors must also include a statement confirming that informed consent must be obtained from all subjects. Authors must certify that their research has been performed in compliance with all institutional, national and international guidelines for human welfare. These statements must be made within the procedure. Referees may be asked to comment specifically on any cases in which concerns arise.

Author contributions statements

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ISSN: The international standard serial number (ISSN) for *Journal of Visualized Experiments* is 1940-087X.

Questions? Please contact your editor or the editorial office (editorial@jove.com).