***Bio-protocol* Manuscript Template**

**Updated September 8, 2022**

This template provides detailed formatting and content instructions for submission to Bio-protocol. If your protocol does not fit our standard template, or if you have suggestions to improve our template and author guidelines (<https://en.bio-protocol.org/protocol_preparation_guidelines.aspx>), contact our Managing Editor, Marisa Rosa ([marisa.rosa@ed.bio-protocol.org](mailto:marisa.rosa@ed.bio-protocol.org)).

**General formatting notes**

1. Click [here](https://en.bio-protocol.org/e2979) to see an example of an article in the standard *Bio-protocol* format.
2. Prepare your manuscript using Microsoft Word.
3. Any English writing conventions (e.g., American English or British English) are acceptable, but the same conventions must be used throughout the manuscript. For example, if you use American English, the spellings for “analyze” and “labeled” should be in the American convention in all cases.
4. Use 10 point Times New Roman for the body of the text.
5. Abbreviations should only be used if necessary and defined upon first use in the Abstract, main text, and each figure, table, or video legend. They can also be repeated in the Reagents, Solutions, and Recipes.
6. **For figures:**
   * 1. For the original submission, embed figures in the text file. Upon acceptance, individual figure files in TIFF format (PMC preferred image format) are required.
     2. Image files should not be manipulated in any way that could result in misinterpreting the original image. Please refer to 'What's in a picture? The temptation of image manipulation' by Rossner and Yamada (Journal of Cell Biology, 166:11) for examples of inappropriate manipulation and valuable guidance on acceptable practice.
     3. Figures should be numbered in the order that they appear in the protocol and placed after the first time they are referenced in the text.
     4. Images should not have layers or a transparent background.
     5. Resolution: minimum of 300 ppi and maximum of 900 ppi.
     6. Color: RGB.
     7. Width: 7.62–14.73 cm.
     8. Text in Figure: 8–12 point (Times New Roman), single spaced. Use the same typeface for all figures.
     9. Include a main title and a comprehensive but concise legend. Include all information needed to understand the figure without having to refer to the main text (e.g., define all abbreviations and symbols used).
     10. Define error bars and statistical tests used to determine significance in the legend.
     11. Include labeled scale bars where appropriate (e.g., microscope images).
     12. Ensure all panels are described in the figure legend.
     13. Panel labels: Use letters “**A**, **B**, **C**,…” for each main panel (uppercase, bold); for subpanels, use “*i*, *ii*, *iii*,…
     14. Use the same typeface for all figures.
7. **For tables within the text of the protocol:**
   1. There are no limits on table size; the *Bio-protocol* editing team will either adjust the size appropriately for optimal online presentation or advise the author to include them as a supplementary file if they are too large.
   2. Prepare tables using the table function of Microsoft Word.
   3. Do not include color or shading in the tables.
   4. Add a label to the top row of each column.
   5. Each table should have a brief title and a comprehensive but concise legend. Include all information needed to understand the table without having to refer to the main text (e.g., define all abbreviations and symbols used).
   6. Place title above the table. Consistently use “**Table 1. Title.** Xxx.”; reference all tables in the main text as “Table X.”.
   7. Tables should have the following three horizontal lines:

One under the title, above the column heading, weight/width = 1 pt;

One between the column headings and the body of the table, weight/width = 0.75 pt;

One at the bottom of the table, weight/width = 1 pt.

* 1. Each table should be inserted below the step or paragraph where it is first cited.

1. **For videos:** 
   * 1. Videos are not embedded in the manuscript but uploaded separately in the *Bio-protocol* submission portal.
     2. A placeholder for each video, including its title and legend, should be included after the first time it is referenced in the text.
     3. Typical smart phone camera videos are usually adequate.
     4. Each video should have a comprehensive but concise legend title and legend. Include all information needed to understand the video without having to refer to the main text (e.g., define all abbreviations and symbols used).
     5. Use standard video formats (e.g., MOV, MPEG, or AVI).
     6. Each video file should be less than 1 GB.
2. **Use the following formats of special symbols and units where it applies:**

Degree Celsius: °C (no space before)

Micro: μ

Alpha: α

Beta: β

Gamma: γ

Less than or equal to: ≤

More than or equal to: ≥

Multiplication: ×

Chemical formula: MgSO4·7H2O

Prime: ′

Liter: L (mL, μL)

Exponentials: Yx

1. **Scientific nomenclature:** The use of standard scientific nomenclature is required. Species, genes, genotypes, and mutations should be italicized. Genetic databases for the species of interest should be consulted to ensure that the recommended names are used. Bio-protocol encourages authors to refer to organisms by their common name (if a common name applies), and to provide the Latin name in parentheses at first use. Authors should determine whether the nomenclature they have used conforms to accepted community standards.

***Bio-protocol* manuscript preparation template**

**Method for Extra- and Intraoral Flavor Delivery in Freely Moving Rats**

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#Contributed equally to this work

**[Abstract]** (one paragraph, maximum 250 words)

Introduce the research field (1-2 sentences), provide context by mentioning other existing and previously used techniques (1-2 sentences), summarize the protocol (3-4 sentences max), and finish by summarizing the advantages of the protocol presented (1-2 sentences). Do not include references (e.g., to articles or figures) in the Abstract.

**[Key Features]** (up to four Key Features, each with a maximum of approximately 25 words)

Key Features are bullet points that convey the most important elements of your protocol. Key features should not simply repeat or condense ideas conveyed in the Abstract. Instead, they should highlight characteristics that will allow readers to determine whether the protocol is relevant to their work. For example, they might mention experimental contexts in which the protocol was developed and is most useful: “This protocol builds upon the method developed by Smith et al., and extends its application to new cell types” or “Optimized for the following organisms”. Key features might also include specific requirements for the protocol, for example: “This protocol requires instrument XX model YY or a more recent model,” or “Requires at least 7 days to complete.” See more examples below.

* Analysis of DNA damage sensitivity using 2-week-old Arabidopsis seedlings.
* Experiments performed *in vitro* for better control of chemical doses applied and increased reproducibility.
* Uses phenotype easy to score by naked eye (number of leaves emerged) as proxy for DNA damage sensitivity.
* Allows testing various doses of DNA damaging agents and various genetic backgrounds simultaneously.

**[Graphical Overview]**

Provide a graphical overview of the procedure in figure format. This component is highly recommended but not required. The overview can be a flowchart of the method/procedures or highlight the key steps.

1. Use a resolution of a minimum of 300 dpi and a maximum of 900 dpi
2. Minimize the use of text in the figure
3. Include a figure legend for clarity, if needed

**Keywords:** Keyword 1, Keyword 2, Keyword 3 …

Provide approximately 5–10 keywords; include technical or method-related keywords and repeat relevant keywords from your original research article, as applicable.

**[****Background]** (maximum 500 words)

Briefly introduce the research area that your protocol can be used to advance. Discuss previously described, related methodologies, and summarize the advantages and limitations of using your protocol over other published methods. If possible, elaborate on other possible applications of the protocol.

**Materials and Reagents**

Organize according to the categories below. List all materials/reagents used in the protocol, including vendor/manufacturer and catalog number. Provide storage information (i.e., storage temperature, shelf-life) as well.

Please include custom-made or lab-made materials, reagents, or equipment (see section below), and provide references or instructions on how to acquire or generate those items. Use consistent nomenclature for all materials/reagents throughout the manuscript.

If you have something that does not fit in the below Materials and Reagent categories, please contact our Managing Editor, Marisa Rosa ([marisa.rosa@ed.bio-protocol.org](mailto:marisa.rosa@ed.bio-protocol.org)).

**Biological Materials**

Include, e.g., bacterial and virus strains, cell lines, or biological samples.

1. Material name 1 (Manufacturer, Brand, catalog number: XXXX or origin)

**Reagents**

1. Product 1 name (Manufacturer, Brand, catalog number: XXXX)
2. Product 2 name (Manufacturer, Brand, catalog number: XXXX)

**Solutions**

1. Solution name (abbreviation if it applies) (See Recipes). Provide recipes for the solutions in the Recipes section below, as needed.

**Recipes**

Be precise about the ingredients (e.g., buffer or media) and quantities used, and the conditions established for your experiments, including storage temperature and shelf-life of each solution. Note that omission of minor details from recipes (such as the type of water used) might lead to the failure of the experiment.

For example:

1. Lysis buffer

|  |  |  |
| --- | --- | --- |
| Reagent | Final concentration | Amount |
| NaCl (5 M) | 150 mM | 3 ml |
| Tris-HCl (1 M, pH 8.0) | 20 mM | 2 ml |
| H2O | n/a | 95 ml |
| Total | n/a | 100 ml |

1. 70% ethanol

|  |  |  |
| --- | --- | --- |
| Reagent | Final concentration | Amount |
| Ethanol (absolute) | 70% | 700 ml |
| H2O | n/a | 300 ml |
| Total | n/a | 1000 ml |

1. 0.02% sodium-hypochlorite (bleach) solution

|  |  |  |
| --- | --- | --- |
| Reagent | Final concentration | Amount |
| Sodium hypochlorite solution (6%) | 0.02% | 3 ml |
| ddH2O | n/a | 897 ml |
| Total | n/a | 900 ml |

**Laboratory Supplies**

List the laboratory supplies used in the experiments, including specific manufacturer catalog numbers; provide references for items that are not commercially available.

1. Product 1 name (Manufacturer, Brand, catalog number: XXXX)

**Equipment**

List the equipment used in the experiments, including specific catalog/model numbers.

1. Equipment 1 name (Manufacturer, Brand, catalog number: XXXX)

**Software and Datasets**

List individual software or datasets used in the experiments, including version and release date. Provide references for items that are not commercially available. Note whether the software is free to use or requires a license, and, if possible, provide free alternatives to commercial software.

1. Item 1 name (version, date)

**Procedure**

1. **Write chronological, step-by-step instructions for the protocol.** We recommend dividing the major steps of the protocol into separate sections (A, B, C…) with descriptive subtitles.
2. **Use up to three list levels in the following order:**

1st Level (section): A. B. C. … [Indentation: Left (0 cm), Hanging (0.63 cm)]

2nd Level (step): 1. 2. 3. … [Indentation: Left (0.63 cm), Hanging (0.63 cm)]

3rd Level (sub-step): a. b. c. … [Indentation: Left (1.26 cm), Hanging (0.63 cm)]

**For example:**

1. Gene cloning
2. Isolate DNA from tissues
   1. Collect sample by (…)
   2. Grind tissue with mortar and pestle (…)
   3. …
3. Set up RT-PCR reaction
4. …
5. Cell transformation
6. Place competent cells on ice (…)
7. Add plasmids to the competent cells (…)
8. …
9. **Write steps as instructions for the reader using the active voice and present tense** (e.g., write "Prepare stock solutions and reaction mixtures under anaerobic conditions” instead of “Stock solutions and reaction mixtures were prepared under anaerobic conditions").
10. **For each step,** include details for material and reagents used (e.g., volume added, specific container/tube, and incubation time) and the conditions in which the step is performed (e.g., temperature, agitation speed, and equipment settings); avoid using vague terms such as “several,” “enough,” “about,” or “around”).
11. **Provide images and videos for key steps, representative data (intermediate and final) to illustrate the type of results obtained, and notes/tips to help others:** Your protocol should provide enough information for a novice to perform successfully with only limited advice or supervision. If special training is required to perform some steps or operate specific equipment, please note this in the protocol and consider mentioning it the Key Features section as well.
12. **Labels and notes to include in the procedure**
    * 1. Mark steps describing safety concerns with “**Caution**” and provide details immediately below.
      2. Mark steps where the protocol can be stopped with “**Pause point**” and explain immediately below.
      3. Mark critical steps with “**Critical**” and explain why immediately below.
      4. Mark any other comments, suggestions, or recommendation with “**Notes**.”
      5. If one of the general notes is relevant to a particular step (but not fundamental to its completion), reference it by number after the step, for example “**see General Note 1**.”
      6. Mark steps where troubleshooting information is available in the **General Notes and Troubleshooting** section with “**see Troubleshooting**.” This should be information on what to do if part or all of the protocol does not work—referencing specific steps, note what can be changed or optimized if particular outcomes happen.

**Data Analysis**

Provide a detailed description of data processing and analyses: include statistical tests, criteria for data inclusion/exclusion, and details on the recommended number of biological and technical replicates in each experiment. Highlight any specific skills necessary to perform the analyses (e.g., expertise using Linux, R) and any specific analysis software.

When a detailed description of the data analysis method already appears in sufficient detail in a published or posted research article, summarize the analysis method and cite the publication or preprint. Please, indicate clearly where the description of the analysis can be found (e.g., Supplemental information or Figure X).

**Validation of Protocol**

Please provide the evidence that this protocol is robust and reproducible. For experimental protocols, include information about the number of replicates, statistical tests applied, and controls that were used to validate the protocol.

Types of Evidence:

1. Reference specific data in a research article published or posted by you or others, e.g., specific figures or supplementary materials in the article or preprint.
2. Provide data directly in this section of your *Bio-protocol* submission.

**General Notes and Troubleshooting**

All notes should be numbered and can be referenced in the text by number if the information is particularly relevant to that step.

**General Notes**

Include comments that apply to the protocol more broadly, not only to individual steps, and are not fundamental to complete a step. For example, notes might address limitations of the protocol, its applicability to other experimental systems and model organisms, or sources of variability.

**Troubleshooting**

State common problems that might occur and describe ways to address them. This information can be provided as a table.

**Acknowledgments**

Include the following information: 1) funding sources; 2) previous work or the original research paper in which this protocol was described and validated.

**Competing interests**

1. The corresponding author should provide a statement of financial and non-financial competing interests on behalf of all authors.
2. Examples include paid employment or consultancy, stock ownership, patent applications, personal relationships with individuals involved in the submission or evaluation of a protocol, and receipt of funding or free products from the vendors of the reagents/equipment or other advertisers. For more examples, see list at [PLOS](http://journals.plos.org/plosmedicine/s/competing-interests).

**Ethical considerations**

1. All protocols that have used human and/or animal subjects must mention the specific ethics committee that approved the described experiment.
2. Protocols including human subjects should also indicate that informed consent was obtained from all subjects.
3. Protocols including clinical trials should clearly state the name of the trial registry and the clinical trial registration number in the manuscript.

**References**

Include all relevant literature in the following format (as used by American Psychological Association 6th edition, no ampersand in author list):

Bindschedler, L. V., Dewdney, J., Blee, K. A., Stone, J. M., Asai, T., Plotnikov, J., Denoux, C., Hayes, T., Gerrish, C., Davies, D. R., Ausubel, F. M., & Bolwell, G. P. (2006). Peroxidase-dependent apoplastic oxidative burst in Arabidopsis required for pathogen resistance. The Plant journal : for cell and molecular biology, 47(6), 851–863. https://doi.org/10.1111/j.1365-313X.2006.02837.x

1. Write the author list, year, title, journal (abbreviated), volume, page range, and DOI.
2. List references alphabetically.
3. In text citations should follow the Harvard author-date style (e.g., Bindschedler et al., 2006).

**Supplementary information**

The following information can be included in this section.

1. Extended data
2. Supplementary information
3. Source data

**If your protocol does not fit this template, or if you have suggestions to improve our template and author guidelines, contact our Managing Editor, Marisa Rosa (**[**marisa.rosa@ed.bio-protocol.org**](mailto:marisa.rosa@ed.bio-protocol.org)**).**