

# Template for a Protocol Article

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## **Abstract**

The abstract should be 250 words in length and provide a summary of the contents of the manuscript. Beneath the abstract paragraph list each of the protocols included in your manuscript and their titles (shown below) – this text counts towards the word limit of your abstract.

Basic Protocol 1: (Title)

Basic Protocol 2: (Title)

etc.

**Keywords:** List 3-5 keywords appropriate to your article.

## **Front matter**

Please submit this Word document to ScholarOne, <https://mc.manuscriptcentral.com/cp>

Every section must be filled for the manuscript to be reviewed.

## **Article Title**

Provide a title for your manuscript. To maximize the discoverability of your article, please see Wiley's Search Engine Optimization (SEO) guidelines [here](#).

## **AUTHOR(S) AND CONTACT INFORMATION:**

List all authors and give contact information— address, phone numbers, email address for each. Denote corresponding author.

## **INTRODUCTION**

Briefly introduce the purpose of the method, what problem(s) it addresses, and what data can be obtained by executing the protocols. Most of the background literature should be cited in the Commentary section (see below). The final paragraph of the introduction should, in sentence form, list all the protocols in the document and provide a brief summary of each one.

### **STRATEGIC PLANNING** (*optional*)

Occasionally a method is sufficiently complex that a Strategic Planning section is required. This section describes, in paragraph form, various procedural options that are essential to know before starting the protocol(s) below.

### **BASIC PROTOCOL 1**

Protocol describing the recommended or most universally applicable approach(es). There can be more than one Basic Protocol. In that case they should be numbered sequentially.

### **BASIC PROTOCOL TITLE**

Provide a title for each protocol.

### **Introductory paragraph**

Briefly introduce protocol. Tell the user what they are doing, how they are doing it and what they should expect if the protocol is conducted properly. Important notes can be added here such as safety hazards, or animal handling guidelines.

### **Materials**

Provide an EXHAUSTIVE list, organized in two sections - the first consisting of reagents, solutions, and starting samples or test organisms/cells, and the second consisting of hardware and instruments (e.g., glassware, disposables, microscopes, centrifuges) used for this protocol, in the order in which they are introduced in the protocol steps below. Names of complex solutions (more than 3 components) and reagents should be listed here – with the recipe details provided in the **Reagents and Solutions** section (the components of those recipes are NOT listed here). Please ensure that enough detail for each material is added such that someone could obtain the exact material listed – depending on the material this might be supplier information, product numbers, CAS numbers, antibody clone numbers, RRIDs, etc.

Anti-CD3 (Bio X Cell, cat. no. BE0001-1, RRID:AB.1107634)  
Anti-CD28 (Bio X Cell, cat. no. BE0015-1, RRID:AB.1107624)  
MACS Naive CD4+orCD8a+T Cell Isolation Kit, mouse (Miltenyi Biotec, cat. no. 130-104-453 or 130-096-543)  
Low Salt Wash Solution (see recipe in Reagents and Solutions)  
HEK 293T cells (ATCC, cat. no. CRL-3216, RRID:CVCL.0063)

Table 1: Example of a detailed materials list

### **Protocol steps — *Step annotations:***

Numbered protocol steps describe the how to perform the protocol. Use the **active voice** (start each sentence with a verb such as weigh, place, measure) and include sufficient detail that the procedure can be successfully completed on the first attempt. Provide any pointers, additional information or explanation for a protocol step as an annotation, directly below the step to which it applies and in italics. Not every step requires an annotation, but authors are strongly encouraged to add annotations to enrich the instructions.

### ***Example of a properly written protocol step with an annotation:***

3. Plate  $1 \times 10^6$  cells/ml in R10 medium with 10 nM OVA257-264SIINFEKL peptide in individual wells of a 24-well or 6-well plate to activate cells.

*Usually during the first 24 h, cell numbers will decrease by 25%. Please recount the cells after the incubation to make sure you use the correct amount in the following step.*

### ***Sample Data***

Provide data in the form of either figures or tables that your lab has generated *FOR EACH PROTOCOL*. This data will act as a benchmark demonstrating to a user exactly what they should achieve if they replicate your experiment correctly. These figures or tables should be cited within the protocol steps – but can also be discussed in Understanding the Results in the Commentary section below. Some protocols, such as growing cells or purifying a peptide, do not produce much data. In these instances, please provide a sufficiently detailed written description of the protocol results such that a user could determine if they have accurately reproduced your steps.

### **Additional protocols**

Use the same structure above for:

1. **Alternate protocols.** Alternate Protocols are used for situations where different equipment or reagents are used to achieve ends similar to the Basic Protocol, where the starting material requires a different approach, or where requirements for the end product differ from the Basic Protocol.

2. **Additional basic protocols.**
3. **Support Protocols.**

## **REAGENTS AND SOLUTIONS:**

Recipes for reagents or solutions that are unique to the protocols described in the manuscript. Provide details on how to produce these recipes and include storage information (temperature and duration). Recipes should be arranged in alphabetical order.

## **COMMENTARY**

### **BACKGROUND INFORMATION:**

Historical background on the development of the technique(s) and/or comparison of the described technique with other methods that may be used for similar analyses.

### **CRITICAL PARAMETERS:**

Factors that influence the protocol and to which special attention should be paid. This section is designed to provide detailed answers to the questions “What parameters should I consider before conducting these protocol(s)? What aspects of this experiment are the most sensitive to the user manipulation? Do certain samples have to be prepared in a special way to obtain reproducible results?” Some authors address each protocol individually, but this section should be written in paragraph form.

### **TROUBLESHOOTING:**

Common problems with the protocols, their causes, and potential solutions. The information may be presented in tabular form or it may be combined with Critical Parameters. This section should answer the follow “What aspects of the protocol are most likely to fail or not reproduce? If a protocol does not work – what are the most common causes and how does one resolve them?”. This section can be written in either paragraph form or entered as a table.

### **STATISTICAL ANALYSIS:** (optional)

When applicable, discuss the most appropriate statistical approaches used to analyze the data. Discuss alternative statistical approaches, if any are available.

## **UNDERSTANDING RESULTS:**

Provide examples of anticipated results that should be obtained using these protocols. For data-rich protocols, please include sample data for each protocol. This data will act as a benchmark for the field demonstrating to a user exactly what they should achieve if they replicate your experiment correctly. If possible, also include negative results and examples of unanticipated findings. This section should provide a thorough discussion of how to interpret the results. It is also acceptable to include sample data at the end of each protocol, if that makes for a clearer presentation of the protocol.

## **TIME CONSIDERATIONS:**

Please describe how long it takes to complete each of the protocols described in the article.

## **ACKNOWLEDGEMENTS: (mandatory for NIH, optional for all others)**

NIH grant-holders must include a funding acknowledgement statement including the NIH grant numbers. All others may acknowledge funding sources or colleague contributions according to their wishes and needs.

## **LITERATURE CITED:**

Bibliographic listing of the references cited in the text. Current Protocols uses APA style and it is picked automatically by Authorea upon export, e.g. (Gallagher, 2018)

References should be listed alphabetically by author's last name. Should you wish to use EndNote or Zotero, please download the corresponding output style on our For Author's Page (<https://currentprotocols.onlinelibrary.wiley.com/hub/forauthors>).

## **KEY REFERENCES: (optional)**

References that are significant for understanding the method but not necessarily cited in the article. Each Key Reference must be accompanied by a short annotation explaining the significance of the reference.

## **INTERNET RESOURCES: (optional)**

URLs for important sites relevant to the method. Each must be accompanied by a short description of the subject of the site.

## **FIGURE LEGENDS:**

Provide legends for each of the figures for the manuscript. **DO NOT INCLUDE THE FIGURES IN THE MANUSCRIPT FILE** . Figures should be submitted as separate high-resolution (266-300 dpi) tif, eps, or jpeg files. Figures may be of any size. If figures are reproduced from a published source, you must acknowledge the source appropriately in the legend and must supply us with documentation showing that permission for use was granted.

## **TABLES: (optional)**

Tables should be added to this document using Insert->Table and uploading a CSV or XLSX file. The table should be editable; not an image of a table.

## **Submission**

The following should be submitted on ScholarOne as individual files, NOT as part of the main document:

- **FIGURES**
- **COPYRIGHT PERMISSION** (if required). If any of the figures or tables have been published previously, please obtain permission to reuse the material from the original copyright holder. Do not include the permission(s) in the manuscript text; rather upload the permission documents as individual files during manuscript submission.
- **VIDEOS** (optional). Videos illustrating how the procedure is accomplished are encouraged. See the Contributor's Style Guide for guidelines to preparing videos. (<https://currentprotocols.onlinelibrary.wiley.com/hub/forauthors>).

## References

Gallagher, S. R. (2018). Recipes for Commonly Encountered Reagents. *Current Protocols Essential Laboratory Techniques*, 17(1), e24. <https://doi.org/10.1002/cpet.24>