Article

Protocol and reagents for pseudotyping lentiviral particles with SARS-CoV-2 Spike protein for neutralization assays and other functional studies

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Received: date; Accepted: date; Published: date

**Abstract:** SARS-CoV-2 enters cells using its Spike protein, which is also the main target of neutralizing antibodies. Therefore, methods to measure how antibodies and sera affect Spike-mediated viral infection are important for studying immunity to SARS-CoV-2. Because actual SARS-CoV-2 is a biosafety-level-3 virus, one way to simplify the study of Spike-mediated infection is to pseudotype Spike on biosafety-level-2 virions. Such pseudotyping has now been described for single-cycle lentiviral, retroviral and VSV virions—but the reagents and protocols are not currently widely available. Here we describe how to effectively pseudotype lentiviral virions with SARS-CoV-2 Spike, and make all the experimental reagents publicly available in the BEI Resources repository of ATCC and the NIH. Furthermore, we show that these pseudotyped virions can be used to measure the neutralizing activity of human sera against SARS-CoV-2 in convenient luciferase-based assays, thereby providing a valuable complement to ELISA-based methods that only measure binding to the virus’s Spike protein.

**Keywords:** SARS-CoV-2, COVID-19, coronavirus, neutralization assay, lentiviral pseudotype, Spike, cytoplasmic tail, ACE2, 293T-ACE2, luciferase

1. Introduction

Describe why pseudotyping is useful and what has been done previously.

2. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

2.1. Subsection

2.2. Figures, Tables and Schemes

All figures and tables should be cited in the main text as Figure 1, Table 1, *etc*.

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| C:\Users\martin\Downloads\testFigure.tif  (**a**) | C:\Users\martin\Downloads\testFigure.tif  (**b**) |

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**Table 1.** This is a table. Tables should be placed in the main text near to the first time they are cited.

|  |  |  |
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| **Title 1** | **Title 2** | **Title 3** |
| entry 1 | data | data |
| entry 2 | data | data 1 |

1 Tables may have a footer.

3.3. Formatting of Mathematical Components

This is an example of an equation:

|  |  |
| --- | --- |
| a = 1, | (1) |

the text following an equation need not be a new paragraph. Please punctuate equations as regular text.

3. Discussion

Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

4. Materials and Methods

Materials and Methods should be described with sufficient details to allow others to replicate and build on published results. Please note that publication of your manuscript implicates that you must make all materials, data, computer code, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited.

Research manuscripts reporting large datasets that are deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

Interventionary studies involving animals or humans, and other studies require ethical approval must list the authority that provided approval and the corresponding ethical approval code.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, Video S1: title.

**Author Contributions:** Conceptualization, K.D.C. and J.DB.; investigation, K.D.C., R.E., A.S.D., K.M., and A.N.L.; resources and specialized reagents, A.B.B. and H.Y.C.; writing—original draft preparation, K.D.C and J.D.B.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research by the following grants from the NIAID of the NIH: R01AI141707 (to J.D.B.) and F30AI149928 (to K.D.C.). J.D.B. is an Investigator of the Howard Hughes Medical Institute.

**Acknowledgments:** We thank Andrew McGuire for helpful suggestions and feedback.

**Conflicts of Interest:** The authors declare no conflict of interest.

References

1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.
2. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196.
3. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, 2008; pp. 154–196.
4. Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* stage of publication   
   (under review; accepted; in press).
5. Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, 2012.
6. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In Title of the Collected Work (if available), Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).
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