




MyCell® Custom iPS Cells User's Guide



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CDI does not in any way guarantee or represent that you will obtain satisfactory results from using MyCell Custom iPS cells as described herein. You assume all risk in connection with your use of MyCell Custom iPS cells.

Conditions of Use

MyCell Custom iPS cells are for life science research use only and subject to the use restrictions as contained in Appendix A. You are responsible for understanding and performing the protocols described within. CDI does not guarantee any results you may achieve. These protocols are provided as CDI's recommendations based on its use and experience with MyCell Custom iPS cells.

Origin

MyCell Custom iPS cells are manufactured in the United States of America.

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Before You Begin

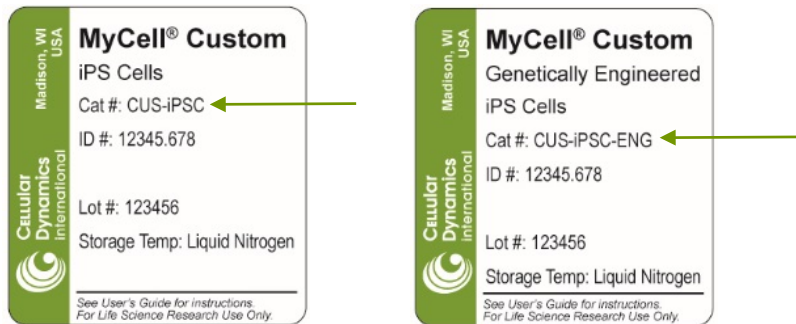
Notes

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire MyCell® Custom iPS Cells User's Guide before handling or using MyCell Custom iPS Cells(Catalog Number CUS-iPSC) or MyCell Custom Genetically Engineered iPS Cells (Catalog Number CUS-iPSC-ENG).
- MyCell Custom iPS cells are for life science research use only. See Appendix A for more information and other restrictions.
- A safety data sheet (SDS) for dimethyl sulfoxide (DMSO), in which MyCell Custom iPS cells are frozen, is available online at www.cellulardynamics.com/lit/ or on request from Cellular Dynamics International. Only technically qualified individuals experienced in handling DMSO and human biological materials should access, use, or handle MyCell Custom iPS cells.

Chapter 1. Introduction

Cellular Dynamics International's (CDI) MyCell Custom iPSC cells are induced pluripotent stem cells reprogrammed from adult somatic cells using CDI's proprietary episomal methodology. These cells were derived from client-provided donor samples exhibiting diverse genotypes and phenotypes. MyCell Custom iPSC cells have the potential to be differentiated into any of the 200+ cell types in the human body and can be used to generate disease models.

This User's Guide is intended for use with both MyCell Custom iPSC Cells (Catalog Number CUS-iPSC) and MyCell Custom Genetically Engineered iPSC Cells (Catalog Number CUS-iPSC-ENG). Following are example labels (1.5X):



Components Supplied by Cellular Dynamics

Notes

Item	Catalog Number
MyCell Custom iPS Cells*	CUS-iPSC
MyCell Custom Genetically Engineered iPS Cells*	CUS-iPSC-ENG
MyCell Custom iPS Cells User's Guide	
Certificate of Testing	
Certificate of Origin	
If required for shipping purposes	

* Safety data sheets are available online at www.cellulardynamics.com/lit/.

Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
37 °C Water Bath	Multiple Vendors	
Biological Safety Cabinet with UV Lamp	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
15 ml Centrifuge Tubes	Multiple Vendors	
6-well Cell Culture Plates	Nunc	140675
Dulbecco's Modified Eagle Medium:Nutrient Mixture F-12 (DMEM/F-12)	Life Technologies	11330-032
Dulbecco's Phosphate Buffered Saline without Ca ²⁺ and Mg ²⁺ (D-PBS)	Life Technologies	14190-144
Essential 8 Medium*	Life Technologies	A14666SA
Growth Factor Reduced Corning Matrigel Matrix (Matrigel)*	Corning	354230
Rho Kinase, H1152	EMD Scientific	555550
mTeSR1*	StemCell Technologies	05857
Pipettes	Multiple Vendors	
Sterile Tissue Culture Grade ddH ₂ O	Multiple Vendors	
Vitronectin*	Life Technologies	A14701SA

* MyCell Custom iPS cells can be maintained in two different cell culture conditions: (1) Essential 8 Medium and vitronectin or (2) mTeSR1 and Matrigel. Order the necessary components accordingly.

Technical Support and Training

MyCell Custom iPS Cells

For inquiries about handling and thawing MyCell Custom iPS cells, contact CDI's Technical Support Scientists:

Telephone (877) 320-6688 (US toll-free) / (608) 310-5100 x5
Monday - Friday, 8:30 am - 5:00 pm US Central Time

Fax (608) 310-5101

Email support@cellulardynamics.com

Essential 8 Medium and Vitronectin

For inquiries about iPS cell culture and growth, contact Life Technologies' Technical Support and refer to materials available online:

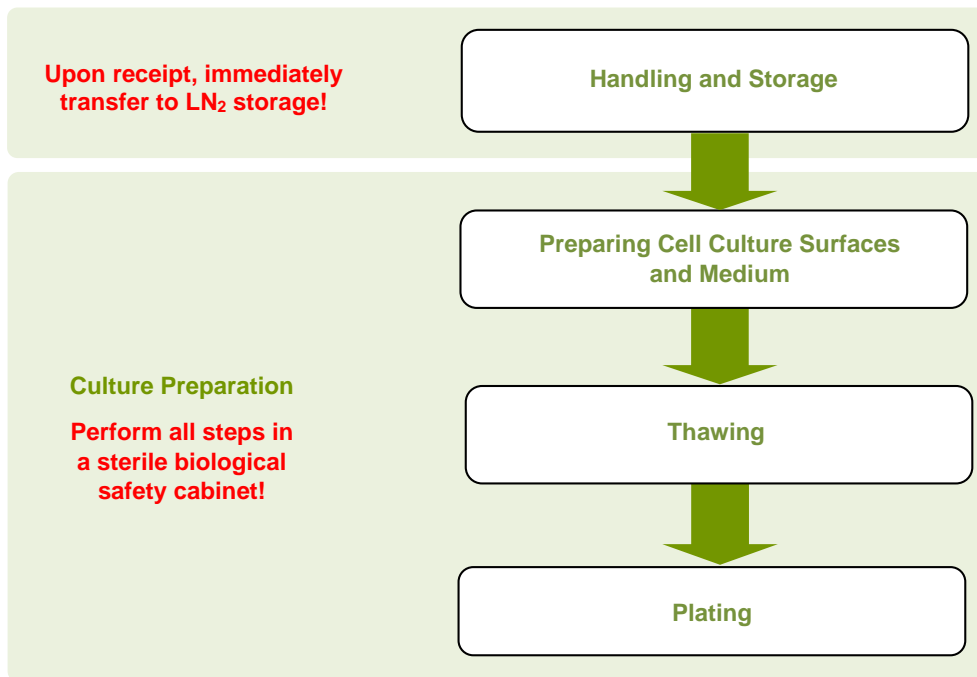
Email techsupport@lifetech.com

Protocols and videos Essential 8 Medium:
<http://products.invitrogen.com/ivgn/product/A14666SA>

Vitronectin:
<http://products.invitrogen.com/ivgn/product/A14701SA>

Workflow Diagram

Notes



Chapter 2. Handling and Storage

MyCell Custom iPS cells are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer the cryobox containing MyCell Custom iPS cells to the vapor phase of a liquid nitrogen cell storage dewar. CDI strongly recommends transferring the entire cryobox into the storage rack to avoid transferring individual vials.



It is critical to maintain cryopreserved MyCell Custom iPS cells at a stable temperature. Minimize exposure of cryopreserved MyCell Custom iPS cells to ambient temperature when transferring vials to liquid nitrogen storage.

Chapter 3. Culturing MyCell Custom iPS Cells in mTeSR1

CDI recommends plating MyCell Custom iPS cells into cell culture plates that are pre-coated with Matrigel and used in conjunction with mTeSR1.

The following sections detail preparing Matrigel and mTeSR1 and thawing MyCell Custom iPS cells using these reagents.



It is critical to perform all subsequent steps in a sterile biological safety cabinet.

Preparing Matrigel Aliquots

1. Equilibrate a sterile box of 200 μ l pipette tips and a sterile container of 1.5 ml tubes at -20°C overnight.
2. Thaw a bottle of Matrigel on ice at 4°C overnight.
3. Calculate the volume of Matrigel required to aliquot 1 mg/tube, which is the amount required to coat a 6-well cell culture plate.

Note: Each lot of Matrigel has a different concentration. Perform the calculation each time a new lot of Matrigel is used.

$$\text{Volume per Tube} = \frac{1 \text{ mg/tube}}{\text{Concentration of Matrigel}}$$

For example, the calculation for a lot of Matrigel with a concentration of 13.841 mg/ml is as follows:

$$\text{Volume per Tube} = \frac{1 \text{ mg/tube}}{13.841 \text{ mg/ml}} = 0.072 \text{ ml}$$

4. Place the 4°C Matrigel, -20°C sterile pipette tips, and -20°C sterile 1.5 ml tubes on ice just before use. Spray the ice container thoroughly with 70% ethanol and place in a biological safety cabinet.
5. Aliquot the calculated volume of Matrigel into 1.5 ml tubes, switching tips when the Matrigel begins to clog the tip and/or the measurement becomes inaccurate. Place the tubes on ice as they are filled.



It is essential to perform this step on ice. Matrigel will solidify at room temperature.

6. Store aliquots at -20°C for up to 1 year.

Preparing the 6-well Cell Culture Plate

1. Add 6 ml of ice-cold DMEM/F-12 to a 15 ml centrifuge tube.
2. Remove an aliquot of Matrigel from -20°C.
3. Immediately add 1 ml of the DMEM/F-12 to the aliquot and mix using a 1 ml pipettor, gently pipetting up and down. Transfer the Matrigel solution to the 15 ml centrifuge tube containing ice-cold DMEM/F-12 and mix to achieve a final concentration of 0.167 mg/ml (1 mg Matrigel/6 ml DMEM/F-12).
4. Add 1 ml/well of Matrigel solution to a 6-well cell culture plate. Scale volumes appropriately for other vessel formats to add Matrigel solution at 0.017 mg/cm².
5. Incubate at room temperature for at least 1 hour before plating MyCell Custom iPS cells.

Note: If necessary, wrap the plate in aluminum foil and store at 4°C for up to 1 week. Incubate plates for 1 hour at room temperature before use.

Preparing mTeSR1

Prepare the mTeSR1 according to the manufacturer's instructions.

Note: If necessary, store mTeSR1 at 4°C for up to 2 weeks.

Preparing mTeSR1 + H1152

1. Prepare a 100 µM stock solution of H1152 in sterile tissue culture grade ddH₂O according to the manufacturer's instructions.
2. Prepare the mTeSR1 + 1 µM H1152 in a 50 ml centrifuge tube by diluting 0.1 ml of 100µM H1152 in 10 ml of mTeSR1.
3. Filter the mTeSR1 + 1 µM H1152 using a 0.2 µm filter.

Note: If necessary, store the 50 ml centrifuge tube containing mTeSR1 + 1 µM H1152 at 4°C, protected from light, for up to 1 week.

Thawing MyCell Custom iPS Cells into mTeSR1

Maintain MyCell Custom iPS cells in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Completing the following steps in a time-efficient manner facilitates optimal MyCell Custom iPS cells viability and performance.

Note: CDI does not recommend thawing more than 1 vial at one time.

1. Equilibrate mTeSR1 and mTeSR1 + H1152 to room temperature, spray with 70% ethanol, and place in a biological safety cabinet.
2. Remove the MyCell Custom iPS cells cryovial from the liquid nitrogen storage tank.

Note: If necessary, place cryovials on dry ice for up to 10 minutes before thawing.

3. Immerse the cryovial in a 37°C water bath (avoid submerging the cap) and gently swirl until only a small piece of frozen material remains (approximately 3 - 5 minutes).

4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the MyCell Custom iPS cells cryovial contents drop-wise to a sterile 15 ml centrifuge tube using a 1 ml pipettor.
6. Rinse the empty MyCell Custom iPS cells cryovial with 1 ml of room temperature mTeSR1 to recover any residual cells from the vial. Transfer the 1 ml of mTeSR1 rinse from the cryovial drop-wise to the 15 ml centrifuge tube containing MyCell Custom iPS cells suspension.
7. Slowly add 8 ml of room temperature mTeSR1 to the 15 ml centrifuge tube containing MyCell Custom iPS cells suspension.
8. Centrifuge the cell suspension at 200 x g for 4 minutes.
9. Aspirate the supernatant to just above the cell pellet.
10. Gently resuspend the cell pellet in 2.5 ml of mTeSR1 + H1152 using a 5 ml serological pipette.

Plating MyCell Custom iPS Cells into a Pre-coated 6-well Cell Culture Plate

1. Aspirate the Matrigel solution from 1 well of a pre-coated 6-well cell culture plate.
2. Immediately add the 2.5 ml of MyCell Custom iPS cells suspension to the well.
3. Evenly distribute the cells by shaking the plate back and forth then side to side.
4. Culture MyCell Custom iPS cells in a cell culture incubator at 37°C, 5% CO₂ for 24 hours.
5. 24 hours post-plating MyCell Custom iPS cells, aspirate the spent mTeSR1 + H1152 and replace with 2.5 ml of mTeSR1.
6. Maintain MyCell Custom iPS cells according to the mTeSR1 manufacturer's instructions.

Chapter 4. Culturing MyCell Custom iPS Cells in Essential 8 Medium (Xeno-free Conditions)

CDI recommends plating MyCell Custom iPS cells into cell culture plates that are pre-coated with vitronectin and used in conjunction with Essential 8 Medium.

The following sections detail preparing vitronectin and Essential 8 Medium, and thawing MyCell Custom iPS cells using these reagents.

Preparing the 6-well Cell Culture Plate

1. Dilute the vitronectin in D-PBS to 5 µg/ml according to the manufacturer's instructions.
2. Add 1 ml/well of vitronectin solution to a 6-well cell culture plate. Scale volumes appropriately for other vessel formats to add vitronectin solution at 0.5 µg/cm².
3. Incubate at room temperature for at least 1 hour before plating MyCell Custom iPS cells.

Note: *If necessary, wrap plates in laboratory film and store at 4°C for up to 1 week. Incubate plates for 1 hour at room temperature before use.*

Preparing Essential 8 Medium

Prepare the Complete Essential 8 Medium according to the manufacturer's instructions.

Note: *If necessary, store Complete Essential 8 Medium at 4°C for up to 2 weeks.*

Preparing Complete Essential 8 + H1152 Medium

1. Prepare a 100 µM stock solution of H1152 in sterile tissue culture grade ddH₂O according to the manufacturer's instructions.
2. Prepare the Complete Essential 8 + 1 µM H1152 medium in a 50 ml centrifuge tube by diluting 0.1 ml of 100 µM H1152 in 10 ml of Complete Essential 8 Medium.
3. Filter the Complete Essential 8 + 1 µM H1152 medium using a 0.2 µm filter.

Note: *If necessary, store the 50 ml centrifuge tube containing Complete Essential 8 + 1 µM H1152 medium at 4°C, protected from light, for up to 1 week.*

Thawing MyCell Custom iPS Cells into Complete Essential 8 Medium

Maintain MyCell Custom iPS cells in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Completing the following steps in a time-efficient manner facilitates optimal MyCell Custom iPS cells viability and performance.

Note: CDI does not recommend thawing more than 1 vial at one time.

1. Equilibrate Complete Essential 8 Medium and Complete Essential 8 + H1152 medium to room temperature, spray with 70% ethanol, and place in a biological safety cabinet.

2. Remove the MyCell Custom iPS cells cryovial from the liquid nitrogen storage tank.

Note: If necessary, cryovials can be placed on dry ice for up to 10 minutes before thawing.

3. Immerse the cryovial in a 37°C water bath (avoid submerging the cap) and gently swirl until only a small piece of frozen material remains (approximately 3 - 5 minutes).

4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.

5. Gently transfer the MyCell Custom iPS cells cryovial contents drop-wise to a sterile 15 ml centrifuge tube.

6. Rinse the empty MyCell Custom iPS cells cryovial with 1 ml of room temperature Complete Essential 8 Medium to recover any residual cells. Transfer the 1 ml of Complete Essential 8 Medium rinse from the cryovial drop-wise to the 15 ml centrifuge tube containing MyCell Custom iPS cells suspension.

7. Slowly add 8 ml of room temperature Complete Essential 8 Medium to the 15 ml centrifuge tube containing MyCell Custom iPS cells suspension.

8. Centrifuge the cell suspension at 200 x g for 4 minutes.

9. Aspirate the supernatant to just above the cell pellet.

10. Gently resuspend the cell pellet in 2.5 ml of Complete Essential 8 + H1152 medium using a 5 ml serological pipette.

Plating MyCell Custom iPS Cells into a Pre-coated 6-well Cell Culture Plate

1. Aspirate the vitronectin solution from 1 well of a pre-coated 6-well cell culture plate.

2. Immediately add the 2.5 ml of MyCell Custom iPS cells suspension to the well.

3. Evenly distribute the cells by shaking the plate back and forth then side to side.

Notes

4. Culture MyCell Custom iPS cells in a cell culture incubator at 37°C, 5% CO₂ for 24 hours.
5. 24 hours post-plating MyCell Custom iPS cells, aspirate the spent Complete Essential 8 Medium + H1152 and replace with 2.5 ml of Complete Essential 8 Medium.
6. Maintain MyCell Custom iPS cells according to the Complete Essential 8 Medium manufacturer's instructions.

Appendix A. Intellectual Property Rights, Use Restrictions, and Limited License

A. **OWNERSHIP.** The MyCell Custom iPS cells and the methods used by CDI to create them are covered by certain patents, pending patents and other intellectual property owned or licensed by CDI: www.cellulardynamics.com/patents. CDI grants Customer a limited, non-transferable license to use the MyCell Custom iPS cells solely for Customer's internal life science research purposes in accordance with the terms of the MyCell Products Agreement under which the MyCell Custom iPS cells were delivered to Customer and subject to the use restrictions in subsection B of this Appendix A and any applicable third party license restrictions or requirements included in subsection C of this Appendix A. No other license or right, express or implied, in or to the MyCell Custom iPS cells or the methods used to create them or any intellectual property owned by or licensed to CDI including the intellectual property embodied in the MyCell Custom iPS cells is conveyed by the delivery of the MyCell Custom iPS cells or the parties' performance under such MyCell Products Agreement. Customer is solely responsible for obtaining any licenses it may require for Customer's specific research use(s) of the MyCell Custom iPS cells. Neither CDI nor its licensor, iPS Academia Japan, Inc. makes any warranty or representation as to the validity, scope, or enforceability of the patents owned by or licensed to CDI as listed at www.cellulardynamics.com/patents.

B. **USE RESTRICTIONS.** Customer shall not propagate more than 120,000,000 live MyCell Custom iPS cells (approximately the number of cells in 10 conventional culture plates) at any one time from any one clone derived from a Donor Sample (i.e., each clone is identified by an unique ID # indicated on the vial label) in all of Customer's facilities worldwide.

The MyCell Custom iPS cells, and any cell directly or indirectly derived or made from the MyCell Custom iPS cells, may be used for Customer's internal life science research purposes only. Customer's use of the MyCell Custom iPS cells also must be in accordance with this User's Guide. Customer may differentiate the MyCell Custom iPS cells using publicly available or its own or licensed differentiation methods. Customer shall not use CDI's patented and/or proprietary methods for differentiation of the MyCell Custom iPS cells, and no license to use such methods is conveyed herein. No right to make, have made, offer to sell, or sell the MyCell Custom iPS cells is implied by the sale or delivery of the MyCell Custom iPS cells. Customer shall not reverse engineer the MyCell Custom iPS cells.

Customer shall not transfer the MyCell Custom iPS cells, or any cell directly or indirectly derived or made from the MyCell Custom iPS cells, to any person including any commercial entity or academic or other similar not-for-profit institution, without first having contacted support@cellulardynamics.com and having obtained CDI's prior written consent or complied with applicable contract requirements.

The MyCell Custom iPS cells, and any cell directly or indirectly derived or made from the MyCell Custom iPS cells, must be used in accordance will all applicable laws and any applicable institutional review board protocol. Customer shall not use the MyCell Custom iPS cells, or any cell directly or indirectly derived or made from the MyCell Custom iPS cells, in humans, in clinical trials, for diagnostic purposes involving human subjects, or for any therapeutic purposes. Customer shall not use the MyCell Custom iPS cells, or any cell directly or indirectly derived or made from the MyCell Custom iPS cells, directly or indirectly to derive or make any human gamete or gamete precursor cell. The MyCell Custom iPS cells, and any cell directly or indirectly derived or made from the MyCell Custom iPS cells, may not be used for services for any third party; nor may they be used in the manufacture of any products. Customer shall not deposit MyCell Custom iPS cells, or any cell directly or indirectly derived or made from the MyCell Custom iPS cells, into any biorepository or any other entity that intends to distribute the MyCell Custom iPS cells, or any cell directly or indirectly derived or made from the MyCell Custom iPS cells.

Notes

Customer will not be entitled to receive any data or information from CDI that directly identifies the donor of biological materials from which the MyCell Custom iPS cells were made; it is the intention that Customer be unable to ascertain readily the identity of such donor and be unable to associate readily the identity of such donor with the data and information provided from Customer's use of the MyCell Custom iPS cells or any cell directly or indirectly derived or made from the MyCell Custom iPS cells. Customer shall not attempt in any way to determine the identity of the donor of the biological materials from which any of the following directly or indirectly are derived or were made: (i) the MyCell Custom iPS cells; or (ii) any cell or other material directly or indirectly derived or made from the MyCell Custom iPS cells.

Appendix B. MyCell Custom iPS Cells Provided "AS IS"

A. The MyCell Custom iPS cells are sold or provided "AS IS."

B. TO THE FULLEST EXTENT PERMITTED BY APPLICABLE LAW, CELLULAR DYNAMICS DISCLAIMS, ALL REPRESENTATIONS, AND WARRANTIES, EXPRESS OR IMPLIED (INCLUDING BUT NOT LIMITED TO, ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE OR NON-INFRINGEMENT), AND ALL LIABILITY, WITH RESPECT TO THE MYCELL CUSTOM iPS CELLS; AND BY ORDERING AND/OR ACCEPTING THE MYCELL CUSTOM iPS CELLS CUSTOMER WAIVES ALL RIGHTS AND REMEDIES OTHERWISE AVAILABLE WITH RESPECT TO THE MYCELL CUSTOM iPS CELLS.

Appendix C. Limited Liability

TO THE FULLEST EXTENT PERMITTED UNDER APPLICABLE LAW, CELLULAR DYNAMICS SHALL NOT HAVE ANY LIABILITY FOR INCIDENTAL, COMPENSATORY, PUNITIVE, CONSEQUENTIAL, INDIRECT, SPECIAL OR OTHER SIMILAR DAMAGES, HOWEVER CAUSED AND REGARDLESS OF FORM OF ACTION WHETHER IN CONTRACT, TORT (INCLUDING NEGLIGENCE), STRICT PRODUCT LIABILITY OR OTHERWISE, EVEN IF CELLULAR DYNAMICS HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. NOTWITHSTANDING ANY OTHER TERM OR IMPLICATION TO THE CONTRARY, UNDER NO CIRCUMSTANCES SHALL CELLULAR DYNAMICS' LIABILITY TO CUSTOMER EXCEED THE AMOUNT PAID BY CUSTOMER FOR THE MYCELL CUSTOM iPS CELLS TO CELLULAR DYNAMICS.

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