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STEMCELL™ TECHNOLOGIES

iCell® Motor Neurons
User's Guide

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CDI does not in any way guarantee or represent that you will obtain satisfactory results from using iCell Motor Neurons as described herein. The only warranties provided to you are included in the Limited Warranty enclosed with this guide. You assume all risk in connection with your use of iCell Motor Neurons.

Conditions of Use

iCell Motor Neurons 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 this guide. CDI does not guarantee any results you may achieve. These protocols are provided as CDI's recommendations based on its use and experience with iCell Motor Neurons.

Origin

iCell Motor Neurons are manufactured in the United States of America.

Copyright Notice

Revision History

Document ID: X1004
Version 1.0: March 2018
Version 0.1: July 2017

Table of Contents

Before You Begin

Notes

- Immediately transfer the frozen vials to liquid nitrogen storage.
- Read this entire iCell® Motor Neurons User's Guide before handling or using iCell Motor Neurons.
- iCell Motor Neurons 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 iCell Motor Neurons 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 iCell Motor Neurons.

Notes

Chapter 1. Introduction

Cellular Dynamics International's (CDI) iCell Motor Neurons are a highly pure population of motor neurons expressing characteristic motor neuron markers. These cells provide a reliable source of human neurons suitable for elucidating the mechanisms of diseases, such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), as well as drug development screening.

When handled and maintained as recommended in this User's Guide, iCell Motor Neurons quickly assume a typical neuronal morphology with branching neurites (Figure 1). In addition, these cells display a stable adherent single-cell morphology and remain viable for an extended culture period (≥14 days) making them amenable to a variety of electrophysiology and mechanistic assays.

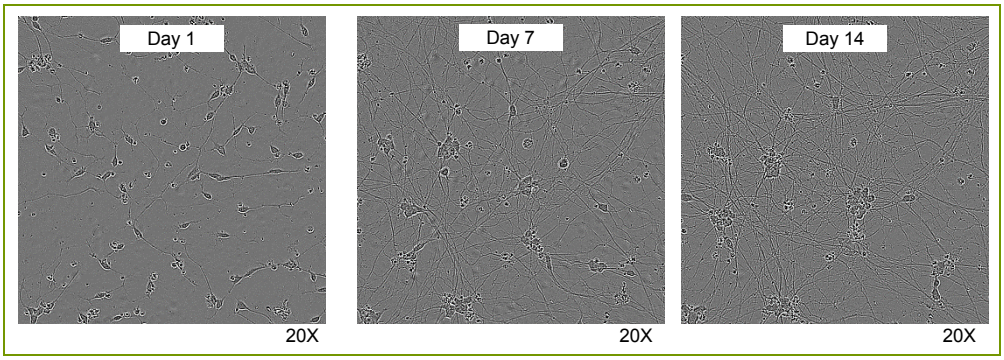

Figure 1: iCell Motor Neurons Exhibit Typical Neuronal Morphology
Brightfield images of iCell Motor Neurons, 01279 at days 1, 7, and 14 post-plating. The re-animated motor neurons develop branched networks within 2 - 3 days and remain viable and adherent for an extended period in culture (≥14 days).

Components Supplied by Cellular Dynamics

Item	Catalog Number	
iCell Motor Neurons Kit, 01279	R1049	R1051
•iCell Motor Neurons, 01279[1, 2]	•C1048 (≥3.0 x 10^6 viable cells)	•C1050 (≥1.0 x 10^6 viable cells)
•iCell Nervous System Supplement[1, 2]	•M1031 (1 ml)	•M1031 (1 ml)
•iCell Neural Base Medium 1[1, 2]	•M1010 (100 ml)	•M1010 (100 ml)
•iCell Neural Supplement A[1, 2]	•M1032 (2 ml)	•M1032 (2 ml)
•iCell Motor Neurons User's Guide[2]	•X1004	•X1004
Certificate of Analysis[3]		
Certificate of Origin If required for shipping purposes		

1 These products were formerly known by these names and/or catalog numbers:
iCell Motor Neurons, 01279 = iCell Motor Neurons (Cat. No. MNC-301-010-001-PT)
iCell Nervous System Supplement = iCell Nervous System Supplement (Cat. No. NSS-301-031-001)
iCell Neural Base Medium 1 = iCell Neurons Maintenance Medium (Cat. No. NRM-100-121-001)
iCell Neural Supplement A = iCell Neurons Medium Supplement (Cat. No. NRM-100-031-001)
Note: *You may receive product labeled with the former name and/or catalog number until current stock is depleted. There were no changes in the manufacture of these cells, media, and supplements.*

2 Safety Data Sheets and User's Guide available online at www.cellulardynamics.com/lit/

3 Available online at www.cellulardynamics.com/coa/

Required Equipment and Consumables

Item	Vendor	Catalog Number
Equipment		
37 °C Water Bath	Multiple Vendors	
Biological Safety Cabinet	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Hemocytometer or Automated Cell Counter[1]	Multiple Vendors	
Liquid Nitrogen Storage Unit	Multiple Vendors	
Pipettors	Multiple Vendors	
Tabletop Centrifuge	Multiple Vendors	
Consumables		
0.22 µm Sterile Filter Unit	Multiple Vendors	
12-well Cell Culture Plates, Poly-D-lysine (PDL) Coated[2]	Multiple Vendors	
6-well Cell Culture Plates, Poly-D-lysine (PDL) Coated[2]	Multiple Vendors	
96-well Cell Culture Plates, Poly-D-lysine (PDL) Coated[2]	Multiple Vendors	
Conical Tubes, 15 ml, Falcon (Centrifuge Tubes)[3]	STEMCELL Technologies	38009
Conical Tubes, 50 ml, Falcon (Centrifuge Tubes)[3]	STEMCELL Technologies	38010
DAPT, ≥98%, Solid	MilliporeSigma	D5942
DMSO, Hybri-Max	MilliporeSigma	D2650
Geltrex Basement Membrane Matrix (Geltrex Matrix)	Thermo Fisher Scientific	A15696-01

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Item	Vendor	Catalog Number
Serological Pipettes, 1, 2, 5, 10, 25 ml[3]	STEMCELL Technologies	38001, 38002, 38003, 38004, 38005
Trypan Blue[3]	STEMCELL Technologies	07050

1 Ensure the automated cell counter is appropriately calibrated before use.
2 Order the format (6-, 12-, or 96-well cell culture plate) required for your experiment.
3 Similar products are available from multiple vendors.

Product and Scientific Support

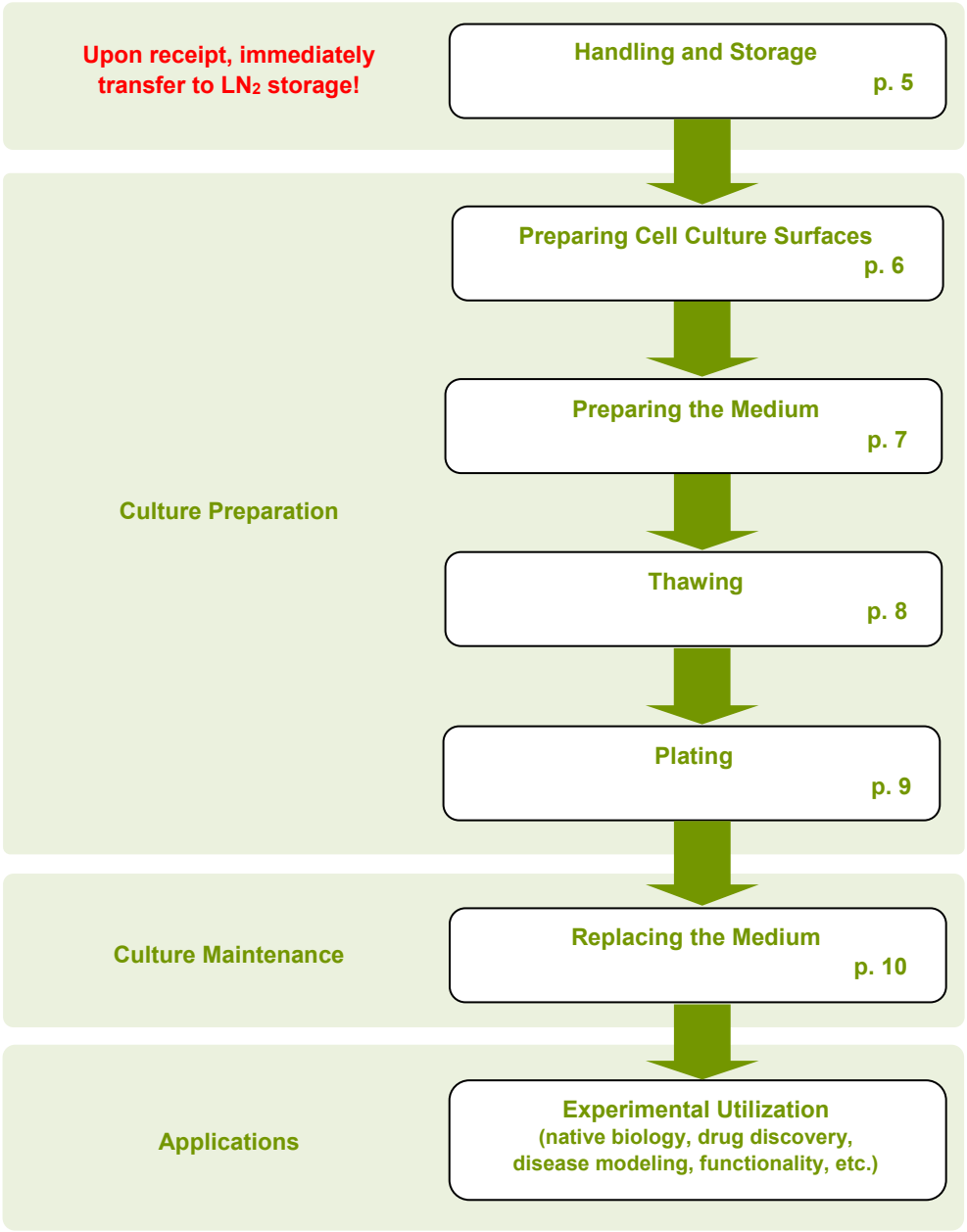
Please contact STEMCELL's Product and Scientific Support for any technical inquiries or questions related to plating, media, cell culture and general assay methods.

Telephone 1 800 667 0322

Email techsupport@stemcell.com

Website www.stemcell.com

Workflow Diagram



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Chapter 2. Handling and Storage

Handling iCell Motor Neurons

iCell Motor Neurons are provided as cryopreserved single-cell suspensions in 1.5 ml cryovials. Upon receipt, directly transfer iCell Motor Neurons to the vapor phase of a liquid nitrogen storage dewar.

It is critical to maintain cryopreserved iCell Motor Neurons at a stable temperature. Minimize exposure of cryopreserved iCell Motor Neurons to ambient temperature when transferring vials to liquid nitrogen storage.

Handling iCell Neuronal Medium and Supplements

iCell Motor Neurons are shipped with three additional components: iCell Neural Base Medium 1 (formerly known as iCell Neurons Maintenance Medium), iCell Neural Supplement A (formerly known as iCell Neurons Medium Supplement), and iCell Nervous System Supplement. iCell Neural Base Medium 1 is shipped at ambient temperature while iCell Neural Supplement A and iCell Nervous System Supplement are shipped frozen on dry ice. Upon receipt, store iCell Base Medium 1 at 4°C and iCell Neural Supplement A and iCell Nervous System Supplement at -20°C until ready for use.

Chapter 3. Preparing Cell Culture Surfaces

Notes

iCell Motor Neurons will plate and function on poly-D-lysine (PDL) coated plates supplemented with a top coating of Geltrex matrix, which are recommended to promote iCell Motor Neurons attachment, long term viability, and function.

Prepare plating surfaces before thawing iCell Motor Neurons.

1. Select the PDL-coated cell culture vessel appropriate for your experimental use.
2. Use the volumes specified in the table below in the following coating procedure. Scale volumes appropriately for other vessel formats.

Culture Vessel	Volume of Geltrex (ml)
6-well Cell Culture Plate	1
12-well Cell Culture Plate	0.8
96-well Cell Culture Plate	0.1

Table 1: Summary of Useful Volumes
*All volumes are **per well**.*

3. Add an appropriate volume of the Geltrex matrix, as specified above, to each well of the vessel(s).
4. Incubate the vessel(s) at room temperature for at least 1 hour.
5. Aspirate the Geltrex matrix immediately before the addition of the cell suspension.

Do not allow the Geltrex matrix-coated surface to dry. Drying of the culture surface can lead to cell clumping and migration.

Notes

Chapter 4. Preparing the Medium

iCell Motor Neurons medium (Complete Maintenance Medium) is comprised of three components: iCell Neural Base Medium 1, iCell Neural Supplement A, and iCell Nervous System Supplement. DAPT should be added to the Complete Maintenance Medium for the first week of culture to prevent outgrowth of proliferative cells. iCell Motor Neurons can be maintained in culture for at least 2 weeks in this medium without appreciable loss of viability or purity.

Complete Maintenance Medium Components	Volume	Final Concentration
iCell Neural Base Medium 1	100 ml	Not Applicable
iCell Neural Supplement A	2 ml	Not Applicable
iCell Nervous System Supplement	1 ml	Not Applicable

Table 2: Volumes for Complete Maintenance Medium Preparation

1. Thaw iCell Neural Supplement A and iCell Nervous System Supplement at room temperature on the day of medium preparation.

   

   Do not thaw the supplements in a 37°C water bath.

2. Spray all medium components with 70% ethanol and place in a biological safety cabinet.
3. Dissolve DAPT in DMSO to achieve a concentration of 20 mM (8.6 mg/ml).
4. Using sterile technique, add iCell Neural Supplement A (~2 ml) and iCell Nervous System Supplement (~1 ml) to iCell Neural Base Medium 1 (~100 ml) to make the Complete Maintenance Medium.
5. Add the Complete Maintenance Medium (50 ml) to two 50 ml centrifuge tubes.
6. Store one tube at 4°C, protected from light, for use during the second week of culture.

   To the other tube, add 12.5 µl of DAPT to achieve a 5 µM final concentration. Filter the Complete Maintenance Medium + DAPT through a 0.22 µm sterile filter unit. Store this medium at 4°C, protected from light, for use during thawing, plating, and the first week of culture.

   Note: *Do not refreeze the individual components of the Complete Maintenance Medium. Complete Maintenance Medium is stable for 2 weeks when stored at 4°C.*

Chapter 5. Thawing iCell Motor Neurons

Notes

Maintain iCell Motor Neurons in liquid nitrogen until immediately before thawing to ensure maximal performance of the cells. Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal iCell Motor Neurons viability and performance.

Note: *Thaw no more than 1 vial of iCell Motor Neurons at one time.*

1. Equilibrate the Complete Maintenance Medium + DAPT at room temperature before thawing iCell Motor Neurons.
2. Remove the iCell Motor Neurons cryovial from the liquid nitrogen storage tank.

   Note: *If necessary, place the cryovial on dry ice for up to 10 minutes before thawing.*
3. Immerse the cryovial in a 37°C water bath for 2 minutes and 30 seconds (avoid submerging the cap), holding the tube stationary (no swirling). Use of a floating microcentrifuge tube rack is recommended.

   

   Precise timing is critical to maximizing viable cell recovery.
4. Immediately remove the cryovial from the water bath, spray with 70% ethanol, and place in a biological safety cabinet.
5. Gently transfer the iCell Motor Neurons cryovial contents to a sterile 50 ml centrifuge tube using a 1 ml pipettor.

   Note: *Use of a 50 ml centrifuge tube facilitates suitable mixing to minimize osmotic shock and increase neuron viability.*

   

   Avoid repeated pipetting of the thawed iCell Motor Neurons cell suspension.
6. Rinse the empty iCell Motor Neurons cryovial with 1 ml of room temperature Complete Maintenance Medium + DAPT to recover any residual cells from the vial. Transfer the 1 ml of Complete Maintenance Medium + DAPT rinse from the cryovial drop-wise (~1 drop/sec) to the 50 ml centrifuge tube containing the iCell Motor Neurons cell suspension. Gently swirl the tube while adding the medium to mix the solution completely and minimize osmotic shock on the thawed cells.

   

   Drop-wise addition of the Complete Maintenance Medium + DAPT to the cell suspension is critical to minimize osmotic shock and ensure maximum viability and attachment.
7. Slowly add 8 ml of room temperature Complete Maintenance Medium + DAPT to the 50 ml centrifuge tube drop-wise (~2 - 3 drops/sec) while gently swirling.

   

   It is critical to add the 8 ml of Complete Maintenance Medium + DAPT slowly to ensure maximum viability and attachment of the cells once plated. Avoid vigorous shaking or vortexing of the cell suspension.

Notes

Chapter 6. Plating iCell Motor Neurons

The recommended plating density for iCell Motor Neurons is 1 x 10^5 viable cells/cm^2 (3.2 x 10^4 cells/well for a 96-well cell culture plate).

1. Transfer the ~10 ml iCell Motor Neurons cell suspension to a 15 ml centrifuge tube.
2. Centrifuge the cell suspension at 400 x g at room temperature for 5 minutes.
3. Carefully aspirate the supernatant, leaving ≥0.5 ml in the centrifuge tube and determine the remaining volume by pipetting.

   Leaving <0.5 ml of medium risks aspirating a portion of the cell pellet.

4. Gently resuspend the cell pellet in 5 ml of room temperature Complete Maintenance Medium + DAPT by flicking the tube and then pipetting up and down 2 - 3 times.

   Avoid excessive pipetting of the cell suspension.

5. Remove a sample of cells to perform a cell count using a hemocytometer (using trypan blue exclusion to identify cells) or an automated cell counter.
6. Dilute the cell suspension with Complete Maintenance Medium + DAPT to obtain a desired cell plating density.
7. Aspirate the Geltrex matrix from the pre-coated cell culture plates and immediately dispense the cell suspension.
8. Culture iCell Motor Neurons in a cell culture incubator at 37°C, 5% CO_2.

Expected Cell Density

iCell Motor Neurons can be plated at various densities to accommodate different applications. However, 1 x 10^5 viable cells/cm^2 is the recommended density for most applications. The following table provides the desired cell number and plating volume for several common cell culture vessels when plating at a density of 1 x 10^5 viable cells/cm^2.

Culture Vessel	Surface Area (cm^2)	Plating Volume (ml)	Cell Number (1 x 10^5 cells/cm^2)
6-well Cell Culture Plate	9.6	2	9.6 x 10^5
12-well Cell Culture Plate	3.8	1	3.8 x 10^5
96-well Cell Culture Plate	0.32	0.2	3.2 x 10^4

Table 3: Summary of Recommended Volumes and Measures
*All volumes and measures are **per well**.*

Chapter 7. Maintaining iCell Motor Neurons

Notes

When plated and maintained in Complete Maintenance Medium, iCell Motor Neurons are able to persist in culture while retaining a high level of purity for an extended culture period (≥14 days).

Complete Maintenance Medium is stable for 2 weeks when stored at 4°C.

1. Immediately before use, equilibrate the Complete Maintenance Medium to room temperature for at least 30 minutes.

   Note: *During the first week in culture, exchange spent medium with Complete Maintenance Medium + DAPT. After the first week, exchange spent medium with only Complete Maintenance Medium.*

   Note: *Do not equilibrate the medium to* 37°C.

   Repeated warming of the Complete Maintenance Medium may decrease stability.

2. Perform a 75% medium exchange on day 2 post-plating with Complete Maintenance Medium + DAPT and then every 2 - 3 days in this manner.

   It is critical to gently dispense the Complete Maintenance Medium to the side of the well to avoid cell detachment.

3. After 1 week in culture, perform a 50% medium exchange with only Complete Maintenance Medium and then every 2 - 3 days in this manner.
4. Culture iCell Motor Neurons in a cell culture incubator at 37°C, 5% CO_2.

Notes

Appendices

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

A. **OWNERSHIP.** The Products are covered by pending patents and patents: cellulardynamics.com/about-us/patents/. Customer has a limited license to use the Products for internal research purposes for the sole benefit of the Customer, subject to the use restrictions included in subsection B of this Appendix A. Customer acknowledges and agrees that the receipt or purchase of the Products by Customer shall not be construed as a transfer of any title or the grant of any rights in or to the intellectual property embodied in the Products owned or licensed by Cellular Dynamics. In particular, no right or license to make, have made, offer to sell, or sell the Products, to modify or reproduce the Product or any part thereof, or to use the Products in combination with any other product(s), except product(s) provided or expressly licensed to Customer by Cellular Dynamics for such use, is implied or conveyed by the sale or transfer of Products to Customer.

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Appendix B. Limited Warranty

A. During the Warranty Period (as defined below) and subject to subsection F of this Appendix B. Cellular Dynamics warrants that its Products conform to the specifications contained in the Certificate of Analysis for the Product shipped to Customer. Customer's sole and exclusive remedy (and Cellular Dynamics' sole and exclusive liability) with respect to any defective Products shall be replacement of the defective Products by Cellular Dynamics pursuant to this Appendix B.

B. Under no circumstances shall Cellular Dynamics' liability to Customer exceed the amount paid by Customer for the Products to Cellular Dynamics. Cellular Dynamics will bear all reasonable shipping costs if the Products are replaced pursuant to this warranty. For clarity, this warranty automatically shall be void, and any claims under it invalid, (i) if Customer's use of the Products is other than solely in accordance with this User's Guide and Cellular Dynamics' Terms and Conditions (or such other written agreement between Cellular Dynamics and Customer under which the Products are sold or transferred to Customer) or for a purpose or in a manner other than that for which the Products were designed; or (ii) if Customer fails to follow this User's Guide for the use, storage, and handling of the Products

however such failure is caused; or (iii) if Customer fails to comply with any of the provisions of Appendix A in this User's Guide; or (iv) if there is any abuse, other misuse or neglect of the Products by Customer or to the extent of any damage or loss of the Products by events or occurrences beyond a person's (e.g., Cellular Dynamics') control including without limitation, accident, fire, vandalism and natural disasters (acts of God). This warranty applies only to Customer and not to third parties. This warranty is not assignable.

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D. Within five (5) business days of thawing the Product but prior to the expiration date of the Product as listed on the Certificate of Analysis and/or Product's label (the "Warranty Period"), Customer must notify Cellular Dynamics in writing of any nonconformity of the Products, describing the nonconformity in detail. Customer's failure to properly notify Cellular Dynamics in the Warranty Period voids the limited warranty set forth above in this Appendix B.

E. Customers who believe they have a warranty claim should call Cellular Dynamics' Technical Support line at (608) 310-5100 ext. 5 or email at support@cellulardynamics.com to request a replacement Product based on a breach of the limited warranty set forth above in this Appendix B. Any action by Customer for Cellular Dynamics' breach of this limited warranty, for which Customer has given timely and proper notice of such breach during the Warranty Period and otherwise in accordance with this Appendix B, must be commenced by Customer within 18 months following the date of such breach.

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G. Customer acknowledges and agrees that Cellular Dynamics may fill Customer's order with any number of units of Products. Such units may be more units than Customer ordered. Customer will not be charged extra for any adjustments made by Cellular Dynamics. The number of cells in a unit is determined by the Product's Certificate of Analysis. The number of cells that are contained in a unit accounts for both viability and plating efficiency percentages. Because this may vary from lot to lot, Cellular Dynamics reserves the right to fill the order with that number of units which is sufficient to fill Customer's order and such adjustments shall not constitute a breach of the limited warranty set forth herein.

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Appendix C. Limited Liability

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