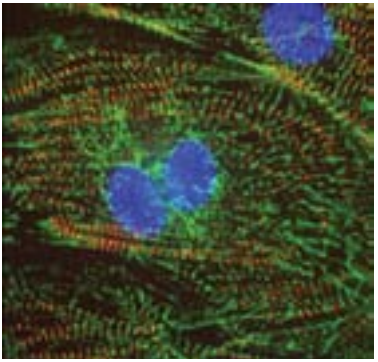
Cytiva™ Plus Cardiomyocytes

Product booklet

Codes:		
	29-0918-80	≥1 × 10^6 (1 000 000) viable cells
	29-0918-81	≥3.5 × 10^6 (3 500 000) viable cells



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1. Legal

License statements

Notice to purchaser: Important license information. The purchase of Cytiva Plus Cardiomyocytes includes a limited license to use the Cytiva Plus Cardiomyocytes for internal research and development, but not for any commercial purposes. Commercial purposes shall include: sale, lease, license or other transfer of the material or any material derived or produced from it; sale, lease, license or other grant of rights to use this material or any material derived or produced from it; use of this material to perform services for a fee for third parties. A license to use the Cytiva Plus Cardiomyocytes for commercial purposes is subject to a separate license agreement with GE Healthcare. IF YOU REQUIRE A COMMERCIAL LICENSE TO USE THIS MATERIAL AND DO NOT HAVE ONE PLEASE CONTACT YOUR LOCAL GE HEALTHCARE REPRESENTATIVE AND RETURN THIS MATERIAL, UNOPENED TO GE HEALTHCARE UK LTD, THE MAYNARD CENTRE, FOREST FARM, WHITCHURCH, CARDIFF CF14 7YT, UK AND ANY MONEY PAID FOR THE MATERIAL WILL BE REFUNDED.

GE Healthcare Cytiva Plus Cardiomyocytes (the Products) are sold under licence from Asterias Biotherapeutics Inc., and Wisconsin Alumni Research Foundation (WARF) under US patent and publication numbers: US 5,843,780, US 6,200,806, US 6,602,711, US 6,800,480, US 7,005,252, US 7,029,913, US 7,297,539, US 7,413,902, US 7,425,448, US 7,452,718, US 7,582,479, US 7,732,199, US 7,763,464, US 7,781,216, US 7,851,167, US 7,897,389 and US 2007/0010012 and equivalent patent and patent applications in other countries.

Important note: For *in vitro* research use only. Not to be used for any therapeutic or diagnostic applications. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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http://www.gelifesciences.com

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2. Handling

A Material Safety Data Sheet (MSDS) for the dimethyl sulphoxide (DMSO) in which Cytiva Plus Cardiomyocytes are frozen, is included with this shipment and available at http://www.gelifesciences.com/msds.

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Cytiva Plus Cardiomyocyte product codes 29-0918-80 and 29-0918-81 are supplied in 1 ml cryovials of $\geq 1 \times 10^6$ (1 000 000) viable cells (1.6×10^6 total cells) and $\geq 3.5 \times 10^6$ (3 500 000) viable cells (5×10^6 total cells) respectively, cryopreserved in 10% DMSO and 90% foetal bovine serum. Upon receipt, the frozen vial(s) of cells should immediately be removed from the outer packaging and transferred to the vapor phase of a liquid nitrogen storage unit at -140°C.

2.3. Expiry

Please refer to the Certificate of Analysis for further details.

2.4. Packaging

Cytiva Plus Cardiomyocytes are provided as cryopreserved single cell suspensions in 1 ml cryovials.

3. Introduction

GE Cytiva Plus Cardiomyocytes are human cardiomyocytes derived from the NIH approved stem cell line NIH hESC-10-0061. Cytiva Plus Cardiomyocytes have been extensively characterized and functionally verified by flow cytometry, sub-cellular imaging and electrophysiology. Cytiva Plus Cardiomyocytes are supplied cryopreserved in a ready to use format.

4. Components and other materials required

4.1. Components

- GE Cytiva Plus Cardiomyocytes
- Certificate of Analysis including product specifications
- Material Safety Data Sheet
- User manual

4.2. Materials to be supplied by user

Product	Supplier/Product code
15 ml Centrifuge tubes	Corning 430052
50 ml Centrifuge tubes	Corning 430290
Serological pipettes – 5 ml	Corning 4487
Serological pipettes – 10 ml	Corning 4488
Serological pipettes – 25 ml	Corning 4489
500 ml Cellulose acetate filter units	Corning 430769
12- or 48-well MEA plate (for MEA applications)	e.g. Axion BioSystems M768-GLx or M768-KAP-48
Multi-well cell culture plates (for HCA and Ca2+ transient applications)	Various e.g. 96-well culture plate (Greiner µClear™) 781091
Multi-well plates for impedance applications	e.g. ACEA E-plate Cardio 96
Coverslips - electrophysiological applications*	VWR 631-0149
1.5 ml tubes	Corning 430290
Sterile bottle (e.g. 30 or 60 ml)	NALG2019-0030/0060

* –not available in North America. Standard glass coverslips of approximately 13 mm diameter and 0.13 mm thickness are suitable.

Product	Supplier/Product code
RPMI 1640 + Glutamine	Gibco 21875034
D-PBS	Sigma D8537
B27 (50×)	Gibco 17504-004
Fibronectin	BD Biosciences 354008
FBS	Gibco 26140-079
Matrigel™	Becton Dickinson 356231
KnockOut™ D-MEM (KO-DMEM)	Gibco 10829-018
Sterile distilled water	Fresenius Kabi 22-96-985

4.3. Equipment needed

Adjustable pipettes and tips
Liquid nitrogen vapour store
Biosafety cabinet
Ice bucket with dry ice
Cryovial rack
Vacuum pump and line
Hemocytometer or automated cell counter
37°C water bath
Centrifuge
Application dependent:
Patch clamp system
Sub-cellular imaging system for High Content Analysis
Multi electrode array system
Impedance system
Calcium transient system

5. Protocols for MEA

5.1. Preparation of MEA coated plates (Time 4 hours)

Consumables

- FBS (at 4°C)
- Sterile distilled H_2O
- Fibronectin (1 mg, human, BD Biosciences 354008) (at 4°C)
- 50 ml tube
- D-PBS

Equipment

- MEA plate (e.g. Axion BioSystems 12 well plate)
- 20 µL pipette & sterile 1-20 µL tips
- 1000 µL pipette & sterile 1-1000 µL tips

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

The first step in preparing an MEA plate for use is to ensure that the surface is hydrophilic. The surface of a new MEA plate is hydrophobic, and even hydrophilic MEAs tend to become hydrophobic again during storage. A hydrophobic surface will prevent attachment and growth of the (hydrophilic) cells. FBS treatment renders the surface hydrophilic.

1. Place a 4 µL bead of FBS over the recording electrode area of each well of the MEA plate (see Fig 1).

Note: If a bead fails to form, ignore that recording site.

2. For a 12 well MEA plate, add 500 µL of sterile distilled H_2O to the gaps between the wells to prevent the evaporation of the bead of FBS (see Fig 1). For a 48 well MEA plate add 200 µL of sterile distilled H_2O to the gaps between the wells.

3. Put the lid on the MEA plate and incubate the MEA plate for 1.5 hours at room temperature.
4. Prepare a 1 mg/ml solution of fibronectin by adding 1 ml sterile distilled H_2O to the 1 mg fibronectin.
5. Take 12.5 µL of this 1 mg/ml fibronectin solution and add it to 987.5 µL D-PBS in a 50 ml tube for a final concentration of 12.5 µg/ml fibronectin.
6. Aspirate the FBS bead from each well of the MEA plate (use a pipette set to dispense 8 µL) and **immediately** replace with a 4 µL bead of 12.5 µg/ml fibronectin solution over the recording electrode area.
7. Put the lid on the tissue culture dish and incubate the MEA plate for 2 hours in a standard cell culture incubator at 37°C.

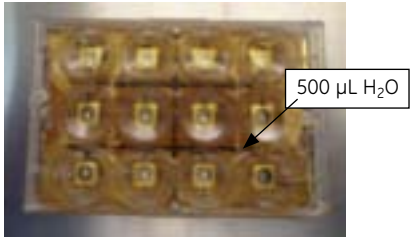

Figure 1. 12 well MEA plate showing location of sterile water addition to the inter-well space to prevent evaporation of the FBS and fibronectin drop.

5.2. Preparation of RPMI 1640/B27 medium for MEA (Time 30 minutes)

Consumables

- RPMI 1640 + Glutamine medium, 500 ml (at 4°C)
- B27™ supplement, 10 ml (at 4°C)
- 10 ml sterile serological pipette

Equipment

- 500 ml 0.22 µm cellulose acetate filter unit (Corning 430769)
- Vacuum line
- Pipette gun

Protocol

During the 2 hour fibronectin incubation step, prepare medium.

Note: Medium may be prepared immediately before thawing Cytiva Plus Cardiomyocytes or prepared and stored at 2–8°C and used within one week of preparation.

1. Thaw frozen 10 ml B27 supplement vial(s) in a 37°C water bath for 10 minutes. Do not incubate at 37°C or expose to light for extended periods of time.

Perform the following steps aseptically inside the biosafety cabinet (BSC).

2. Wipe the required number of RPMI 1640 + Glutamine medium bottle(s) and B27 Supplement vial(s) with 70% isopropanol and transfer to a BSC.
3. Place the filtration unit into the BSC.
4. Using a 10 ml sterile serological pipette, add 10 ml of B27 supplement to a 500 ml bottle of RPMI1640 + Glutamine medium. Swirl bottle several times to mix.
5. Carefully transfer the medium and supplement into the reservoir of the filter unit.

6. Place the lid on the filter unit.
7. Connect the filter unit to a vacuum source.
8. When filtration is complete, disconnect the vacuum source.
9. Detach the upper reservoir of the filtration unit.
10. Place sterile cap on the bottle portion of the filter unit.

Note: Store the medium at 2–8°C. Use within one week of preparation. Avoid repeated warming of the RPMI 1640/B27 medium. Warm only the required volume of medium to complete the task.

5.3. Thawing Cytiva Plus Cardiomyocytes for MEA (Time 30 minutes)

Consumables

- 3.5 × 10^6 vial of Cytiva Plus Cardiomyocytes
- RPMI 1640/B27 medium (filter sterilized)
- 50 ml tube
- 10 ml sterile serological pipette

Equipment

- Ice bucket with dry ice
- Cryovial rack
- 37°C water bath
- Centrifuge
- 1000 µL pipette & sterile 1-1000 µL tips
- Pipette gun

Protocol

During the 2 hour fibronectin incubation step, perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Remove the cryovial from the cryostore and place onto dry ice until ready to thaw.

2. Thaw cell suspension in a 37°C water bath with gentle agitation until ice crystals just disappear.

Note: Take care not to immerse the whole cryovial into the water bath. Avoid extended incubation at 37°C.

3. Wipe the outside of the cryovial with 70% isopropanol and transfer to BSC.
4. Carefully transfer the cell suspension into a sterile 50 ml centrifuge tube using a 1000 µL pipette.
5. Rinse the inside of the cryovial with 1 ml of room temperature RPMI 1640/B27 and combine with the cell suspension drop-wise with gentle mixing.
6. Slowly (over the course of 2 minutes) add 8 ml of RPMI 1640/B27 to the 50 ml centrifuge tube.
7. Centrifuge at 300 g for 5 minutes at 20°C.
8. Carefully, using a 10 ml stripette remove 8 mls of the supernatant. Remove a further 1 ml with a 1000 µL pipette taking care not to disturb the cell pellet. Resuspend the cells in the residual liquid (approx. 1 ml) with gentle agitation.

5.4. Determining Post-Thaw Cell Viability of Cytiva Plus Cardiomyocytes for MEA (Time 30 minutes)

During the 2 hour fibronectin incubation step, determine the viable cell number and viable cell density using preferred method of choice. A method for the NucleoCounter™ NC-100™ (ChemoMetec) cell counter is outlined below. However, this method could be adapted for other commercial cell counters.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 4 × 1.5 ml tubes
- 4 × NucleoCassettes™ (ChemoMetec)
- Reagent A100 (ChemoMetec)
- Reagent B (ChemoMetec)

Equipment

- 100 µL pipette & sterile 1-200 µL tips
- 1000 µL pipette & sterile 1-1000 µL tips
- NucleoCounter NC-100 (ChemoMetec)

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Determine viable cell number using the NucleoCounter NC-100 (ChemoMetec). To do this, transfer 40 µL of the cell suspension into a 1.5 ml tube. Add to the tube 360 µL of RPMI 1640/B27 medium (i.e. now a 1:10 dilution). Gently agitate to achieve an even cell suspension.
2. The remaining steps can be performed outside of the BSC.
3. Transfer 100 µL of 1:10 diluted cell suspension into each of three more 1.5 ml tubes.
4. Use a 100 µL of 1:10 diluted cell suspension sample to calculate the number of Non-Viable Cells/ml (i.e. directly load the sample into a NucleoCassette).
5. Repeat step 4 for a second time, and calculate the average result.
6. Use another 1 × 100 µL of 1:10 diluted cell suspension sample to calculate the Total Number of Cells/ml. To do this, add 100 µL of Reagent A100, and then 100 µL of Reagent B, to the 100 µL of 1:10 diluted cell suspension sample. Mix by pipetting, then load into a NucleoCassette.

7. Repeat step 6 for a second time, and calculate the average result.
8. Determine the total Volume of Cell Suspension.

Note: We use a 1000 µL pipette to establish this volume.

9. Calculate the number of viable cells in the cell suspension:

$$\text{Viable cells/ml} = 10 \times [(3 \times \text{Total Number of Cells/ml}) - (\text{Non-Viable Cells/ml})]$$

$$\text{Total number of viable cells} = \text{Viable cells/ml} \times \text{Volume of Cell Suspension (ml)}$$

Worked example for a typical 3.5 × 10^6 vial of Cytiva Plus Cardiomyocytes:

Total Number of Cells/ml determined in step 6 = 1.83×10^5

Non-Viable Cells/ml determined in step 5 = 1.64×10^5

$$\text{Viable cells/ml} = 10 \times [(3 \times 1.83 \times 10^5) - (1.64 \times 10^5)] = 3.85 \times 10^6 \text{ cells/ml}$$

$$\text{Total number of viable cells} = 3.85 \times 10^6 \text{ cells/ml} \times 1 \text{ ml} = 3.85 \times 10^6 \text{ cells}$$

Note: There are a total of 5.5×10^6 cells in a 3.5×10^6 vial of Cytiva Plus Cardiomyocytes and we routinely record a value of >70% post thaw cell viability (i.e. 3.85×10^6 viable cells).

5.5. Seeding Cytiva Plus Cardiomyocytes onto MEA plates (Time 3 hours)

Cells should be seeded at a density of 6×10^4 viable cells in 4 µL (i.e. 1.5×10^7 viable cells/ml) over the recording electrode area.

Note: Assuming the volume of the cell suspension is ~1 ml, and the number of viable cells is 3.85×10^6, the current concentration is ~3.85×10^6 viable cells/ml. Consequently the next step involves centrifuging and resuspending the cells from section 5.4 to achieve the appropriate cell concentration.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 50 ml tube
- 60 ml bottle

Equipment

- Fibronectin coated well MEA plate
- 20 µL pipette & sterile 1-20 µL tips
- 200 µL pipette & sterile 1-200 µL tips
- 1000 µL pipette & sterile 1-1000 µL tips

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Warm 25 ml RPMI 1640/B27 medium in a 60 ml bottle (sealed) in a 37°C water bath.
2. Centrifuge the cell suspension in a 50 ml tube at 300 g for 5 minutes at 20°C.
3. Carefully, using a 1000 µL pipette, remove the supernatant.
4. Resuspend the cells in the residual liquid with gentle agitation.
5. Determine the total volume of the cell suspension.

Note: We use a 100 µL pipette to establish this volume.

6. Dilute the cell suspension to 1.5×10^7 viable cells/ml using warm RPMI 1640/B27 medium.

$$\text{Required volume (ml)} = \frac{\text{Viable cells}}{1.5 \times 10^7 \text{ viable cells/ml}}$$

Note: Assuming number of viable cells is 3.85×10^6, the volume should be made up to 260 µL.
Remove fibronectin-coated well MEA plate from incubator.

8. Aspirate the fibronectin bead from each well of the MEA plate (use a pipette set to dispense 8 µL) and **immediately** replace with a 4 µL bead of Cytiva Plus Cardiomyocyte suspension (1.5×10^7 viable cells/ml) over the recording electrode area (see Fig 2).

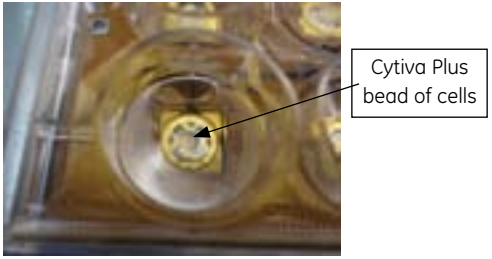

Figure 2. Fibronectin bead replaced with Cytiva Plus cardiomyocyte bead.

9. After seeding all wells, put the lid on the tissue culture dish and incubate the MEA plate in a standard cell culture incubator at 37°C for 2–3 hours. The sterile distilled water in the gaps between the wells will prevent the evaporation of the bead of cell suspension.

10. After 2–3 hours, gently add 500 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 12-well MEA plate or 150 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 48-well MEA plate. Pipette against the side of the well so as not to disturb the plated cells.
11. Gently add another 500 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 12-well MEA plate or 150 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 48-well MEA plate.
12. Gently add another 1000 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 12-well MEA plate (i.e. total well volume is now 2 ml) or another 150 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 48-well MEA plate (i.e. total well volume is now 450 µL).
13. Aspirate the 500 µL of sterile distilled H_2O from the gaps between the wells in the 12-well MEA plate or 200 µL of sterile distilled H_2O from the gaps between the wells in the 48-well MEA plate.
14. Confirm cell attachment at this point by observing the MEA wells under the microscope if using a transparent plate (see Fig 3A and 3B).
15. Incubate in a standard cell culture incubator at 37°C, 5% CO_2.

5.6. Media change on day 4 post-thaw (Time 20 minutes)

1. Warm 30 ml RPMI 1640/B27 medium in a 60 ml bottle (sealed) in a 37°C water bath.
2. Perform the following steps aseptically inside the biosafety cabinet (BSC).
3. For the 12-well MEA plate, using a 1000 µL pipette, carefully aspirate 1000 µL of media from each well of the 12-well MEA plate, then gently add 1000 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 12-well MEA plate, to give a total volume of 2 ml.
4. For the 48-well plate, using a 1000 µL pipette, carefully aspirate 250 µL of media from each well of the 48-well MEA plate then using a 200 µL pipette, gently add 200 µL of warm RPMI 1640/B27 medium to the 'corner' of each well of the 48-well MEA plate, to give a total well volume of 400 µL.
5. Perform MEA recordings 5–7 days after plating onto the MEA plate. It is the responsibility of the user to determine the optimal culture time for this application.

3A

3B

Figure 3A – Wells containing Cytiva Plus Cardiomyocytes and media.
Figure 3B – Cytiva Plus Cardiomyocytes as viewed under a microscope, 5 days after seeding.

6. Protocols for HCA

6.1. Preparation of cell culture plates for HCA (Time 2½ hours)

Consumables

- Sterile distilled H_2O
- Fibronectin (1 mg, human, BD Biosciences 354008) (at 4°C)
- 50 ml sterile tube
- D-PBS (sigma D8537)

Equipment

- 384–well cell culture plate (e.g. Greiner µClear 781091)
- 96-well cell culture plate (e.g. Greiner µClear 655090)
- 20 µL pipette & sterile 1–40 µL tips
- 1000 µL pipette & sterile 1–1000 µL tips

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Prepare a 1 mg/ml solution of fibronectin by adding 1 ml sterile distilled water to the 1 mg fibronectin.
2. Take 125 µL of this 1 mg/ml fibronectin solution and add it to 9875 µL D-PBS in a 50 ml tube for a final concentration of 12.5 µg/ml fibronectin. Add 100 µL of 12.5 µg/ml fibronectin solution to each well of the 96–well cell culture plate, or 30 µL to each well of a 384–well cell culture plate.
3. Put the lid on the cell culture plate and incubate for 2 hours in a standard cell culture incubator at 37°C, 5% CO_2.

6.2. Preparation of RPMI 1640/B27 medium for HCA (Time 20 minutes)

Consumables

- RPMI 1640 + Glutamine medium, 500 ml (at 4°C)
- B27 supplement, 10 ml (at -20°C)
- 10 ml sterile serological pipette

Equipment

- 500 ml 0.22 µm cellulose acetate filter unit (Corning 430769)
- Vacuum line
- Pipette gun

Protocol

During the 2 hour fibronectin incubation step, prepare medium.

Note: Medium may be prepared immediately before thawing Cytiva Plus Cardiomyocytes or prepared and stored at 2–8°C and used within one week of preparation.

1. Thaw frozen 10 ml B27 supplement vial(s) for 10 minutes in a 37°C water bath. Do not incubate at 37°C or expose to light for extended periods of time.

Perform the following steps aseptically inside the biosafety cabinet (BSC).

2. Wipe the required number of RPMI 1640 + Glutamine medium bottle(s) and B27 Supplement vial(s) with 70% isopropanol and transfer to a BSC.
3. Place the filtration unit into the BSC.
4. Using a 10 ml sterile serological pipette, add 10 ml of B27 supplement to 500 ml bottle of RPMI 1640 + Glutamine medium. Swirl bottle several times to mix.
5. Carefully pour 510 ml of the RPMI 1640/B27 medium into the reservoir of the filtration unit.

6. Place the lid on the filter unit.
7. Connect the filter unit to a vacuum source.
8. When filtration is complete, disconnect the vacuum source.
9. Detach the upper reservoir of the filtration unit.
10. Place sterile cap on the bottle portion of the filter unit.

Note: Store the medium at 2–8°C. Use within one week of preparation. Avoid repeated warming of the RPMI 1640/B27 medium. Warm only the required volume of medium to complete the task.

6.3. Thawing Cytiva Plus Cardiomyocytes for HCA (Time 30 minutes)

Consumables

- 3.5 × 10^6 vial of Cytiva Plus Cardiomyocytes
- RPMI 1640/B27 medium (filter sterilized)
- 50 ml sterile tube
- 10 ml sterile serological pipette

Equipment

- Ice bucket with dry-ice
- Cryovial rack
- 37°C water bath
- Centrifuge
- 1000 µL pipette & sterile 1-1000 µL tips
- Pipette gun

Protocol

During the 2 hour fibronectin incubation step, perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Remove the cryovial from the cryostore and place onto dry-ice until ready to thaw.

2. Thaw cell suspension in a 37°C water bath with gentle agitation until ice crystals just disappear.

Note: Take care not to immerse the whole cryovial into the water bath. Avoid extended incubation at 37°C.

3. Wipe the outside of the cryovial with 70% isopropanol and transfer to BSC.
4. Carefully transfer the cell suspension into a sterile 50 ml centrifuge tube using a 1000 µL pipette.
5. Rinse the inside of the cryovial with 1 ml of room temperature RPMI 1640/B27 and combine with the cell suspension drop-wise with gentle mixing.
6. Slowly (over the course of 2 minutes) add 8 ml of RPMI 1640/B27 to the 50 ml centrifuge tube.
7. Centrifuge at 300 g for 5 minutes at 20°C.
8. Carefully, using a 10 ml stripette remove 8 mls of the supernatant. Remove a further 1 ml with a 1000 µL pipette taking care not to disturb the cell pellet. Resuspend the cells in the residual liquid (approx. 1 ml) with gentle agitation.

6.4. Determining Post-thaw Cell Viability of Cytiva Plus Cardiomyocytes for HCA (Time 30 minutes)

During the 2 hour fibronectin incubation step, determine the viable cell number and viable cell density using preferred method of choice. We use a NucleoCounter NC-100 (ChemoMetec) cell counter, the method for which is outlined below. However, this method could be adapted for other commercial cell counters.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 4 × 1.5 ml tubes
- 4 × NucleoCassettes (ChemoMetec)
- Reagent A100 (ChemoMetec)
- Reagent B (ChemoMetec)

Equipment

- 100 µL pipette & sterile 1–200 µL tips
- 1000 µL pipette & sterile 1–1000 µL tips
- NucleoCounter NC-100 (ChemoMetec)

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Determine viable cell number using the NucleoCounter NC-100 (ChemoMetec). To do this, transfer 40 µL of the cell suspension into a 1.5 ml tube. Add to the tube 360 µL of RPMI 1640/B27 medium (i.e. now a 1:10 dilution). Gently agitate to achieve an even cell suspension.
2. The remaining steps can be performed outside of the BSC.
3. Transfer 100 µL of 1:10 diluted cell suspension into 4 × 1.5 ml tubes.
4. Use a 100 µL of 1:10 diluted cell suspension sample to calculate the number of Non-Viable Cells/ml (i.e. directly load the sample into a NucleoCassette).
5. Repeat step 4 for a second time, and calculate the average result.
6. Use another 1 × 100 µL of 1:10 diluted cell suspension sample to calculate the Total Number of Cells/ml. To do this, add 100 µL of Reagent A100, and then 100 µL of Reagent B to the 100 µL of 1:10 diluted cell suspension sample. Mix by pipetting, then load into a NucleoCassette.

7. Repeat step 6 for a second time, and calculate the average result.
8. Determine the total Volume of Cell Suspension.

Note: We use a 1000 µL pipette to establish this volume.

9. Calculate the number of viable cells in the cell suspension:

$$\text{Viable cells/ml} = 10 \times [(3 \times \text{Total Number of Cells/ml}) - (\text{Non-Viable Cells/ml})]$$

$$\text{Total number of viable cells} = \text{Viable cells/ml} \times \text{Volume of Cell Suspension (ml)}$$

Worked example for a typical 3.5×10^6 vial of Cytiva Plus Cardiomyocytes:

Total Number of Cells/ml determined in step 6 = 1.83×10^5

Non-Viable Cells/ml determined in step 5 = 1.64×10^5

$$\text{Viable cells/ml} = 10 \times [(3 \times 1.83 \times 10^5) - (1.64 \times 10^5)] = 3.85 \times 10^6 \text{ cells/ml}$$

$$\text{Total number of viable cells} = 3.85 \times 10^6 \text{ cells/ml} \times 1 \text{ ml} = 3.85 \times 10^6 \text{ cells}$$

Note: There are a total of 5.5×10^6 cells in a 3.5×10^6 vial of Cytiva Plus Cardiomyocytes and we routinely record a value of >70% post thaw cell viability (i.e. 3.85×10^6 viable cells).

6.5. Seeding Cytiva Plus Cardiomyocytes into cell culture plates for HCA (Time 30 minutes)

Cells should be seeded at a density of 3.6×10^4 viable cells in 200 µL RPMI 1640/B27 per well of a 96–well cell culture plate, or 9×10^3 viable cells in 50 µL RPMI 1640/B27 per well of a 384–well cell culture plate (i.e. 1.8×10^5 viable cells/ml).

Note: Assuming the volume of the cell suspension is ~1 ml, and the number of viable cells is 3.85×10^6, the current concentration is ~3.85×10^6 viable cells/ml. Consequently the next step involves diluting the cell suspension to achieve the appropriate cell concentration.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 60 ml bottle

Equipment

- Fibronectin coated cell culture plate
- 200 µL pipette & sterile 1-200 µL tip

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Warm 25 ml RPMI 1640/B27 medium in a sterile 60 ml bottle (sealed) in a 37°C water bath.
2. Dilute the cell suspension to 1.8×10^5 viable cells/ml using warm RPMI 1640/B27 medium.

$$\text{Required volume (ml)} = \frac{\text{Viable cells}}{1.8 \times 10^5 \text{ viable cells/ml}}$$

Example: A total viable count of 3.85×10^6 cells would be divided by 1.8×10^5 viable cells/ml to obtain a final required volume of 21.4 ml.

3. Remove fibronectin-coated cell culture plate from the incubator.
4. Aspirate the fibronectin from each well of the cell culture and **immediately** replace with 200 µL of Cytiva Plus Cardiomyocyte suspension (i.e. 1.8×10^5 viable cells/ml) per well of a 96-well cell culture plate, or 50 µL per well of a 384-well cell culture plate.
5. After seeding all the required wells, put the lid on the cell culture plate and incubate in a standard cell culture incubator at 37°C, 5% CO_2.

6.6. Media change on day 4 post-thaw (Time 20 minutes)

1. Warm 30 ml RPMI 1640/B27 medium in a 60 ml bottle (sealed) in a 37°C water bath.
2. Perform the following steps aseptically inside the biosafety cabinet (BSC).
3. After 96 hours, flick the media out of the plate onto clean tissues and replace with 40 µL of warmed RPMI 1640/B27 medium into each well so as not to disturb the seeded cells. Incubate plate at 37°C, 5% CO_2.
4. Repeat a media change at day 7 replacing the seeded medium with fresh pre-warmed RPMI 1640/B27 medium.
5. Perform HCA 7–8 days after seeding in cell culture plates. It is the responsibility of the user to determine the optimal culture time for this application.

Figure 4 – Brightfield images of Cytiva Plus Cardiomyocytes seeded in microplates for HCA after 8 days post thaw.

7. Protocols for Manual Patch Clamp

7.1. Sterilization of glass coverslips (Time 4 hours)

Consumables

- Borosilicate glass coverslips (13 mm)
- Isopropanol
- 24-well cell culture plate
- 50 ml sterile tube

Equipment

- Sterile forceps

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Sterilize the coverslips by soaking in isopropanol for a minimum of 2 hours in a sealed sterile 50 ml tube. Ensure complete wetting of both sides of each coverslip.
2. Using sterile forceps remove a coverslip from the isopropanol and shake the excess isopropanol from the coverslip. Place the coverslip into the well of a sterile 24-well plastic cell culture plate.
3. Repeat steps 1–3 for the number of wells required.
4. Let the coverslips air-dry in a BSC for a minimum of 2 hours.

7.2. Preparation of 1:2 diluted Matrigel aliquots (Time 10 minutes with O/N thaw)

Consumables

- Matrigel (BD Biosciences 356231, -20°C)
- KO-DMEM (2–8°C)
- 10 ml sterile serological pipette (2–8°C)
- 10 × 50 ml sterile tubes (2–8°C)

Equipment

- Pipette gun

Protocol

Do not allow the Matrigel solution to reach room temperature. Keep the solution, pipettes and KO-DMEM cold at all stages of handling. Avoid repeated freeze-thawing of diluted Matrigel aliquots. Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Slowly thaw Matrigel at 2–8°C to avoid the formation of a gel.

Note: This process is usually performed overnight.

2. Cool a sterile 10 ml serological pipette by drawing and releasing 10 ml of cold KO-DMEM into the pipette repeatedly without removing the pipette from the bottle of KO-DMEM.
3. Add 10 ml of cold KO-DMEM to the vial containing 10 ml Matrigel.
4. Working quickly, mix the Matrigel and KO-DMEM with a 10 ml pipette, avoiding the formation of bubbles.
5. Aliquot 2 ml of diluted Matrigel into each pre-chilled sterile 50 ml tube; store at -20°C until required. Diluted Matrigel solution is stable for 3 months when stored at -20°C.

7.3. Preparation of 1:30 diluted Matrigel (Time 10 minutes)

Consumables

- 2 ml 1:2 diluted Matrigel (-20°C)
- KO-DMEM (2–8°C)
- 5 ml sterile serological pipette (2–8°C)
- 25 ml sterile serological pipette (2–8°C)

Equipment

- Pipette gun

Protocol

Do not allow the Matrigel solution to reach room temperature. Keep the solution and pipettes cold at all stages of handling. Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Slowly thaw a 2 ml 1:2 diluted Matrigel aliquot prepared in step 7.2 at 4°C for at least 2 hours to avoid the formation of a gel. Once thawed transfer to the BSC.
2. Cool a sterile 5 ml serological pipette by drawing 5 ml of cold KO-DMEM into the pipette.
3. Dilute the 2 ml 1:2 diluted Matrigel aliquot with 5 ml cold KO-DMEM. Carefully mix Matrigel solution, avoiding the formation of bubbles.
4. Cool a sterile 25 ml pipette by drawing 25 ml of cold KO-DMEM into the pipette.
5. Add a further 23 ml cold KO-DMEM (for a final dilution of 1:30). Carefully mix Matrigel solution, avoiding the formation of bubbles.

7.4. Preparation of Matrigel-coated coverslips (Time 10 minutes)

Consumables

- 30 ml 1:30 diluted Matrigel (4°C)
- 24-well cell culture plate containing glass coverslips
- KO-DMEM (2–8°C)
- 10 ml sterile serological pipette (2–8°C)
- 10 × 50 ml tubes

Equipment

- Pipette gun

Protocol

Do not allow the Matrigel solution to reach room temperature. Keep the solution, pipettes and KO-DMEM cold at all stages of handling. Avoid repeated freeze-thawing of diluted Matrigel aliquots. Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Add 400 µL of 1:30 diluted Matrigel to each well of the 24-well cell culture plate containing a glass coverslip.
2. Incubate the cell culture plate overnight at 2–8°C before use.
3. Matrigel coated vessels are stable for 10 days when stored at 2–8°C.

7.5. Preparation of RPMI 1640/B27 medium for manual patch clamp (Time 20 minutes)

Consumables

- RPMI 1640 + Glutamine medium, 500 ml (at 4°C)
- B27 supplement, 10 ml (at 4°C)
- 10 ml sterile serological pipette

Equipment

- 500 ml 0.22 µm cellulose acetate filter unit (Corning 430769)
- Vacuum line
- Pipette gun

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Using a 10 ml sterile serological pipette, add 10 ml of B27 supplement to 500 ml bottle of RPMI 1640 + Glutamine medium. Swirl bottle several times to mix.
2. Place the filtration unit into the BSC.
3. Carefully pour 510 ml of the RPMI/B27 medium into the reservoir of the filtration unit.
4. Place the lid on the filter unit.
5. Connect the filter unit to a vacuum source.
6. When filtration is complete, disconnect the vacuum source.
7. Detach the upper reservoir of the filtration unit.
8. Place sterile cap on the bottle portion of the filter unit.

Note: Store the medium at 2–8°C. Use within one week of preparation. Avoid repeated warming of the RPMI 1640/B27 medium. Warm only the required volume of medium to complete the task.

7.6. Thawing Cytiva Plus Cardiomyocytes for manual patch clamp (Time 30 minutes)

Consumables

- 24-well cell culture plate containing Matrigel-coated coverslips (2–8°C)
- 1 × 10^6 vial of Cytiva Plus Cardiomyocytes
- RPMI 1640/B27 medium (filter sterilized)
- 50 ml sterile tube
- 10 ml sterile serological pipette

Equipment

- Ice bucket with dry-ice
- Cryovial rack
- 37°C water bath
- Centrifuge
- 1000 µL pipette & sterile 1–1000 µL tips
- Pipette gun

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Before thawing the cryovial of cells, take the 24-well cell culture plate containing the Matrigel-coated coverslips out of 2–8°C storage. Leave the 24-well cell culture plate at room temperature for 1 hour before seeding the cells.
2. Remove the cryovial from the cryostore and place onto dry-ice until ready to thaw.
3. Thaw cell suspension in a 37°C water bath with gentle agitation until ice crystals just disappear.

Note: Take care not to immerse the whole cryovial into the water bath. Avoid extended incubation at 37°C.

4. Wipe the outside of the cryovial with 70% isopropanol and transfer to BSC.
5. Carefully transfer the cell suspension into a sterile 50 ml centrifuge tube using a 1000 µL pipette.
6. Rinse the inside of the cryovial with 1 ml of room temperature RPMI 1640/B27 and combine with the cell suspension drop-wise with gentle mixing.
7. Slowly (over the course of 2 minutes) add 8 ml of RPMI 1640/B27 to the 50 ml centrifuge tube.
8. Centrifuge at 300 g for 5 minutes at 20°C.
9. Carefully, using a 10 ml stripette remove 8 mls of the supernatant. Remove a further 1 ml with a 1000 µL pipette taking care not to disturb the cell pellet. Resuspend the cells in the residual liquid (approx. 1 ml) with gentle agitation.

7.7. Determination of post-thaw viability of Cytiva Plus Cardiomyocytes for manual patch clamp (Time 30 minutes)

Determine the viable cell number and viable cell density using preferred method of choice. We use a NucleoCounter NC-100 (ChemoMetec) cell counter, the method for which is outlined below. However, this method could be adapted for other commercial cell counters.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 4 × 1.5 ml tubes
- 4 × NucleoCassettes (ChemoMetec)
- Reagent A100 (ChemoMetec)
- Reagent B (ChemoMetec)

Equipment

- 100 µL pipette & sterile 1–200 µL tips
- 1000 µL pipette & sterile 1–1000 µL tips
- NucleoCounter NC-100 (ChemoMetec)

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Determine viable cell number using the NucleoCounter NC-100 (ChemoMetec). To do this, transfer 40 µL of the cell suspension into a 1.5 ml tube. Add to the tube 360 µL of RPMI 1640/B27 medium (i.e. now a 1:10 dilution). Gently agitate to achieve an even cell suspension.
2. The remaining steps can be performed outside of the BSC.
3. Transfer 100 µL of 1:10 diluted cell suspension into each of three more 1.5 ml tubes.
4. Use a 100 µL of 1:10 diluted cell suspension sample to calculate the number of Non-Viable Cells/ml (i.e. directly load the sample into a NucleoCassette).
5. Repeat step 4 for a second time, and calculate the average result.
6. Use another 1 × 100 µL of 1:10 diluted cell suspension samples to calculate the Total Number of Cells/ml. To do this, add 100 µL of Reagent A100, and then 100 µL of Reagent B, to the 100 µL of 1:10 diluted cell suspension sample. Mix by pipetting, then load into a NucleoCassette.
7. Repeat step 6 for a second time, and calculate the average result.
8. Determine the total Volume of Cell Suspension.

Note: We use a 1000 µL pipette to establish this volume.

9. Calculate the number of viable cells in the cell suspension:

$$\text{Viable cells/ml} = 10 \times [(3 \times \text{Total Number of Cells/ml}) - (\text{Non-Viable Cells/ml})]$$

$$\text{Total number of viable cells} = \text{Viable cells/ml} \times \text{Volume of Cell Suspension (ml)}$$

Worked example for a typical 1.0 × 10^6 vial of Cytiva Plus Cardiomyocytes:

Total Number of Cells/ml determined in step 6 = 5.33×10^4

Non-Viable Cells/ml determined in step 5 = 4.80×10^4

$$\text{Viable cells/ml} = 10 \times [(3 \times 5.33 \times 10^4) - (4.80 \times 10^4)] = 1.12 \times 10^6 \text{ cells/ml}$$

$$\text{Total number of viable cells} = 1.12 \times 10^6 \text{ cells/ml} \times 1 \text{ ml} = 1.12 \times 10^6 \text{ cells}$$

Note: There are a total of 1.6×10^6 cells in a 1.0×10^6 vial of Cytiva Plus Cardiomyocytes and we routinely record a value of >70% post thaw cell viability (i.e. 1.12×10^6 viable cells).

7.8. Seeding Cytiva Plus Cardiomyocytes onto coverslips (Time 30 minutes)

Cells should be seeded at a density of 4.6×10^4 viable cells in 650 µL RPMI 1640/B27 per well of a 24-well cell culture plate µL (i.e. 7.1×10^4 viable cells/ml).

Note: Assuming the volume of the cell suspension is ~1 ml, and the number of viable cells is 1.12×10^6, the current concentration is ~1.12×10^6 viable cells/ml. Consequently the next step involves diluting the cell suspension to achieve the appropriate cell concentration.

Consumables

- RPMI 1640/B27 medium (filter sterilized)
- 60 ml bottle

Equipment

- 24-well cell culture plate containing Matrigel-coated coverslips
- 200 µL pipette & sterile 1-200 µL tips
- 37°C water bath

Protocol

Perform the following steps aseptically inside the biosafety cabinet (BSC).

1. Warm 25 ml RPMI 1640/B27 medium in a sterile 60 ml bottle (sealed) in a 37°C water bath.
2. Dilute the cell suspension to 7.1×10^4 viable cells/ml using warm RPMI 1640/B27 medium.

$$\text{Required volume (ml)} = \frac{\text{Viable cells}}{7.1 \times 10^4 \text{ viable cells/ml}}$$

Example: A total viable count of 1.12×10^6 cells would be divided by 7.1×10^4 viable cells/ml to obtain a final required volume of 15.8 ml.

3. Aspirate the Matrigel from each well of the 24-well cell culture plate and **immediately** replace with 650 µL of Cytiva Plus Cardiomyocyte suspension (i.e. 7.1×10^4 viable cells/ml).
4. After seeding all the required wells, put the lid on the cell culture plate and incubate in a standard cell culture incubator at 37°C, 5% CO_2.

7.9. Media change on day 4 post-thaw (Time 20 minutes)

1. Warm 30 ml RPMI 1640/B27 medium in a 60 ml bottle (sealed) in a 37°C water bath.
2. Perform the following steps aseptically inside the biosafety cabinet (BSC).
3. After 96 hours, carefully aspirate off 300 µL of the medium from each well, leaving 350 µL residual medium and replace with 300 µL of warmed RPMI 1640/B27 medium so as not to disturb the seeded cells. Incubate plate at 37°C, 5% CO_2.
4. Repeat a media change at day 7 replacing half the seeded medium with fresh pre-warmed RPMI 1640/B27 medium.
5. Perform manual patch clamp 4–7 days after seeding in cell culture plates. It is the responsibility of the user to determine the optimal culture time for this application.

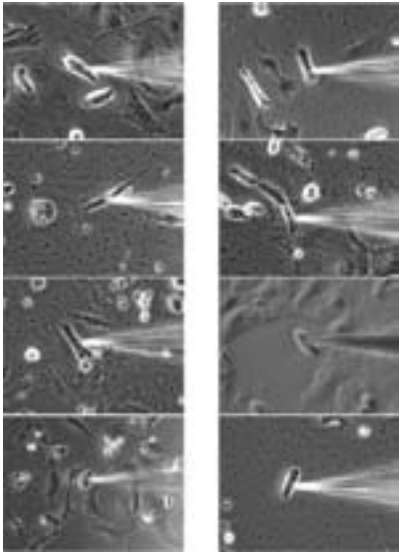

Figure 5 – Examples of cell morphologies patched manually.

8. Troubleshooting

Problems	Solutions
Poor cardiomyocyte viability	Check storage/shipping conditions. Follow pack leaflet to revive cells from cryostore. Follow recommended procedure for thawing and dilution of cardiomyocytes as improper handling of Cytiva Plus Cardiomyocytes could cause low viability. Ensure pre-warmed medium is added drop-wise to the cardiomyocytes.
Poor cell attachment	Make sure plate being used is tissue culture treated and sterile. Follow recommended procedure for preparation and storage of fibronectin plates. Aspirate fibronection just before seeding. Be careful not to touch the bottom of plate. Make sure the surface of the well has not been marked during the removal of fibronectin. Do not let the plate dry out. Place into incubator on a flat surface for 2 hours. Make sure viable count number is used to seed.

Problems	Solutions
Poor cell attachment	Follow feeding steps in pack leaflet. Ensure RPMI 1640/B27 is warmed immediately before use and avoid prolonged exposure to light. Use RPMI 1640/B27 within 7 days of preparation and avoid repeated warming. Ensure that Matrigel coverage is uniform by microscopic inspection. Matrigel coated plates should be warmed to room temperature for at least 30 minutes before use.
Uneven coating of Matrigel	Ensure culture vessels are stored on a level surface. Use only cell culture treated plastic ware.
Aggregation of Matrigel	Follow recommended procedure for preparation of Matrigel coating of plates. Avoid warming of Matrigel – keep on ice during processing if necessary. Avoid room temperature plastic coming in contact with Matrigel.

Problems	Solutions
Poor ion channel activity	Allow at least 72 hour post thaw before use depending on the application. Ion channel activity will increase as cells recover from thaw. Ensure correct seeding density of Cytiva Plus for the application.
Poor performance on MEA	Do not allow the droplets of cell suspension (5.5.9) to dry out. The cell droplet should still be visible post 2–3 hour incubation prior to adding medium to the well (5.5.10). Take care when adding the medium to the well so as not to disturb the seeded cells (5.5.10). If using re-usable MEA plates ensure that the MEA plates are: (A) Sterile and the plating surface is dry and free of cellular debris (B) Maintained and stored as directed by the manufacturer

9. Related Products

Product name	Code
Cytiva Cell Health Assay for high-content analysis	29-0244-68
Cytiva Cell Integrity Assay for high-content analysis	29-0244-69
Hardware	**Code**
IN Cell Analyzer 2200 Imaging System	29-0278-86
IN Cell Analyzer 6000 Imaging System	28-9939-14
Options	**Code**
IN Cell Analyzer Transmitted Light Module	28-9534-87
IN Cell Analyzer Temperature Control Module	28-9534-73
IN Cell Analyzer Environmental Control Module	28-9534-85
IN Cell Analyzer Liquid Handling Module	28-9798-62
Software	**Code**
IN Cell Investigator, single seat license	28-4089-71
IN Cell Investigator Zebrafish Analysis, software plug-in	28-9826-95
IN Cell Miner HCM, single academic use	28-9624-55
IN Cell Miner HCM, single commercial use	28-9624-56

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28-0918-80 AA 04-2014

Cytiva Plus Cardiomyocytes User Guide

Product protocol card

Codes: 29-0918-80 ≥1 × 10^6 (1 000 000) viable cells
29-0918-81 ≥3.5 × 10^6 (3 500 000) viable cells

Thaw vial of cardiomyocytes at 37°C. Remove vial contents to a 50 ml tube with a 1 ml pipette.

↓

Rinse vial with 1 ml RPMI 1640/B27 and slowly add to the cardiomyocytes

↓

Add a further 8 mls of RPMI 1640/B27 to the cell suspension

↓

Centrifuge cell suspension at 300 g for 5 minutes

↓

Remove supernatant and resuspend cell pellet in RPMI 1640/B27. Perform a cell count

↓

Dilute cell suspension to required cell density

↓

Dispense into appropriate cell culture device

↓

Incubate at 37°C for 4–7 days depending on application.
Feed 4 days post thaw and every 3 days thereafter with RPMI 1640/B27.

Warning: *For research use only*.
Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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