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GETTING STARTED

Please make sure to read this entire Pluricyte® Cardiomyocyte Manual carefully before you start to thaw the Pluricyte® Cardiomyocytes.

Pluricyte® Cardiomyocytes are for *in vitro* life science research use only. See Appendix A for general terms and conditions of sale.

A Material Safety Data Sheet (MSDS) for Pluricyte® Cardiomyocytes is available online at www.pluriomics.com/safety.

TECHNICAL SUPPORT AND TRAINING

Our scientists are ready to help you with any questions you may have regarding this manual or the Pluricyte® Cardiomyocytes. In addition, in-lab training is available upon request. For further information please visit our website www.pluriomics.com, or contact us directly by e-mail (support@pluriomics.com).

IMPORTANT RECOMMENDATIONS

- Carefully follow the thawing and seeding instructions, this step is essential for optimal cell survival and attachment (Section 3.2).
- This manual describes the thawing and culture procedures of Pluricyte® Cardiomyocytes on regular tissue culture plates. For plating Pluricyte® Cardiomyocytes on specific MEA-plates, including E-plates® (ACEA Biosciences), 48-well MEA plates (Axion Biosystems), or 6/-1-well MEAs (Multi Channel Systems), we kindly refer you to our assay-specific application notes available online (www.pluriomics.com/support/application-protocols/).
- We strongly recommend to use fibronectin as coating substrate for MEA plates and to use Matrigel™ or fibronectin coating substrate for standard tissue culture plates. Other types of coating substrates may impact the condition of the cells.
- Always refresh the Pluricyte® Cardiomyocyte Medium (PCM) of the cells the day after seeding the cells (Section 3.3). Subsequently, refresh the PCM of the cells every 2 days, or 3 days when refreshing at Friday and Monday to prevent weekend work.
- First contractions of Pluricyte® Cardiomyocytes appear between 24-48 hours post-thaw. It will take 3-4 days before the cells have formed an electrically coupled monolayer. Stable beating monolayers can be observed 7-8 days post-thaw. The optimal time window to perform electrophysiology-based assays with Pluricyte® Cardiomyocytes is between 8-12 days after plating the cardiomyocytes.
1. INTRODUCTION

Pluricyte® Cardiomyocytes are fully functional human induced pluripotent stem cell (hiPSC) derived ventricular cardiomyocytes that are particularly suitable for predictive electrophysiology- and contractility assays for safety pharmacology, toxicity testing and efficacy screening in early drug discovery. Pluricyte® Cardiomyocytes are cultured in Pluricyte® Cardiomyocyte Medium (PCM), which is a serum-free and chemically defined medium designed to promote cardiomyocyte maturation and cell function. Pluricyte® Cardiomyocytes can be maintained in culture for at least two weeks post-thaw.

Pluricyte® Cardiomyocytes strengths and characteristics
Pluricyte® Cardiomyocytes exhibit a relatively high level of maturity, when compared to other human stem cell-derived cardiomyocytes and present the following unique characteristics:

- High purity of ventricular cardiomyocytes
- Low resting membrane potentials (~78 mV)
- Fast upstroke velocities and action potential amplitudes
- Organized sarcomeric structures
- Monolayer field potential contains well-pronounced depolarization and repolarization peaks, enabling easy detection of field potential durations in MEA assays

This manual describes the thawing and culture procedures of Pluricyte® Cardiomyocytes on regular tissue culture plates (different formats).
### 2. EQUIPMENT, MATERIALS AND REAGENTS

**Table 1. Equipment, materials and reagents**

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow cabinet</td>
<td>Various</td>
</tr>
<tr>
<td>Incubator at 37°C, with 5% CO2 and humidified air</td>
<td>Various</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Various</td>
</tr>
<tr>
<td>P10 pipette</td>
<td>Various</td>
</tr>
<tr>
<td>P20 pipette</td>
<td>Various</td>
</tr>
<tr>
<td>P1000 pipette</td>
<td>Various</td>
</tr>
<tr>
<td>Multichannel pipette 30-300μl (when plating on 96-well plates)</td>
<td>Various</td>
</tr>
<tr>
<td>Pipette controller</td>
<td>Various</td>
</tr>
<tr>
<td>Optional: Dry-ice in foam box</td>
<td>Various</td>
</tr>
<tr>
<td>Optional: Foam float for in water bath</td>
<td>Various</td>
</tr>
<tr>
<td>Optional: water bath at 37°C</td>
<td>Various</td>
</tr>
</tbody>
</table>

**Materials**

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>sterile disposable 5 ml pipettes</td>
<td>Various</td>
</tr>
<tr>
<td>sterile 50 ml conical tubes</td>
<td>Various</td>
</tr>
<tr>
<td>sterile filter tips for p10 pipette</td>
<td>Various</td>
</tr>
<tr>
<td>sterile filter tips for p20 pipette</td>
<td>Various</td>
</tr>
<tr>
<td>sterile filter tips for p1000 pipette</td>
<td>Various</td>
</tr>
<tr>
<td>sterile filter tips multichannel (when plating on 96-well plates)</td>
<td>Various</td>
</tr>
<tr>
<td>sterile tissue culture plates, flat bottom, clear plates, TC treated</td>
<td>Various</td>
</tr>
<tr>
<td>sterile multichannel reservoirs (when plating on 96-well plates)</td>
<td>Various</td>
</tr>
<tr>
<td>Parafilm™</td>
<td>Various</td>
</tr>
</tbody>
</table>

**Reagents**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pluricyte® Cardiomyocytes</td>
<td>Plurionics Cat#PCM1-xxxx</td>
</tr>
<tr>
<td>Pluricyte® Cardiomyocyte Medium</td>
<td>Plurionics Cat# PM-2100-100 mL</td>
</tr>
<tr>
<td>1x DPBS + Ca^{2+} + Mg^{2+} *</td>
<td>Gibco Cat#14040</td>
</tr>
<tr>
<td>Fibronectin (1 mg/ml) *</td>
<td>Sigma F1141</td>
</tr>
<tr>
<td>DMEM/F12*</td>
<td>Gibco Cat#31331</td>
</tr>
<tr>
<td>Matrigel™ hESC-qualified Matrix*</td>
<td>Corning Cat#354277</td>
</tr>
</tbody>
</table>

*We strongly recommend to use fibronectin as coating substrate for MEA plates and to use Matrigel™ or fibronectin coating substrate for standard tissue culture plates. Other types of coating substrates may impact the condition of the cells.*
3. METHODS

3.1 Coating of tissue culture plates

For cardiomyocyte adhesion, plastic ware needs to be coated before plating the cells. We strongly recommend to use fibronectin as coating substrate for MEA plates and to use Matrigel™ or fibronectin coating substrate for standard tissue culture plates. Other types of coating substrates may impact the condition of the cells.

3.1.1 Coating of plastic ware with fibronectin

Fibronectin is a biological matrix preparation used to coat plastic ware in order to enable cardiomyocytes adhere to the surface.

1. Dilute fibronectin 1:100 in D-PBS (incl. Ca²⁺/Mg²⁺) to get a 10 μg/ml fibronectin coating solution. Mix the solution carefully.  
   *Note: fibronectin is susceptible to shear stress, do not vortex or spin the solution, and avoid harsh pipetting.*

2. Plate the coating solution immediately onto plates (see Table 2 for recommended coating solution volumes).
   Incubate the fibronectin-coated plate in a cell culture incubator at 37°C, with 5% CO₂ for 3 hours.  
   *Note: longer incubation times are acceptable, however, the fibronectin coating solution should not dry out; this causes irreversible loss of extracellular matrix properties.*

3. Aspirate excess fibronectin coating solution right before plating the cells (see section 3.2 for thawing and plating the Pluricyte® Cardiomyocytes).

<table>
<thead>
<tr>
<th>Plate format</th>
<th>Volume to plate per well</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well</td>
<td>0.05 ml</td>
</tr>
<tr>
<td>48-well</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>24-well</td>
<td>0.50 ml</td>
</tr>
<tr>
<td>12-well</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>6-well</td>
<td>2.00 ml</td>
</tr>
<tr>
<td>T25 flask</td>
<td>5.00 ml</td>
</tr>
</tbody>
</table>
3.1.2 Coating of plastic ware with Matrigel™

Matrigel™ is a biological matrix preparation used to coat plastic ware in order to enable cardiomyocytes adhere to the surface.

*Note: Matrigel™ polymerizes above 10°C. Keep all reagents and final coating solution at 4°C until use.*

1. Thaw an aliquot of Matrigel™ 1:100 in cold DMEM/F12 on ice into a 50 ml conical tube following Manufacturer’s protocol.
2. Mix the diluted Matrigel™ coating solution carefully.
3. Plate the Matrigel™ coating solution immediately onto the plates (see Table 3 for recommended coating solution volumes) and allow for polymerization for at least 45 min at room temperature.
   *Note: never let the Matrigel™ coating solution dry out as this causes irreversible loss of extracellular matrix properties.*
4. Aspirate excess Matrigel™ coating solution right before plating the cells (see section 3.2 for thawing and plating the Pluricyte® Cardiomyocytes).

<table>
<thead>
<tr>
<th>Plate format</th>
<th>Volume to plate per well</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well</td>
<td>0.10 ml</td>
</tr>
<tr>
<td>48-well</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>24-well</td>
<td>0.50 ml</td>
</tr>
<tr>
<td>12-well</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>6-well</td>
<td>2.00 ml</td>
</tr>
<tr>
<td>T25 flask</td>
<td>5.00 ml</td>
</tr>
</tbody>
</table>

Table 3. Recommended Matrigel™ coating solution volumes per well.
3.2 Thawing Pluricyte® Cardiomyocytes

This part of the protocol describes the thawing of Pluricyte® Cardiomyocytes (stored in vapor phase of liquid nitrogen). Complete the following steps of the thawing procedure in a time-efficient manner to facilitate optimal viability and performance.

*Note: the volumes used below are calculated for 1 Pluricyte® Cardiomyocyte vial. If more cells are needed, combine the contents of the vials in the 50 ml conical tube (see step 4) and adjust the volumes of Pluricyte® Cardiomyocyte Medium (PCM) to add accordingly. We recommend to thaw maximum 3 vials per operator at a time.*

1. Coat the tissue culture plate(s) with fibronectin or Matrigel™ coating solution as described in [section 3.1](#).
2. Warm 6 ml PCM to room temperature (RT).
   *Note: make sure to mix the medium by inverting before use.*
3. Take 1 vial of Pluricyte® Cardiomyocytes from LN₂ storage (optional: transport the vial on dry ice) and place the vial in a 37°C incubator for exactly 4 minutes.
4. Gently transfer the contents of the vial to a 50 ml tube using a p1000 pipet. Avoid pipetting up and down.
5. Rinse the empty vial with 1 ml PCM (RT) and add the 1 ml PCM drop-wise to the 50 ml tube containing the cells: add 1 drop every 5 seconds using a p1000 pipette while gently swirling the cells after each drop.
   *This step is crucial for the recovery of the cardiomyocytes. We recommend to use a timer.*
6. Add 4.7 ml of PCM drop-wise to the 50 ml tube, 1 drop every 2 seconds using a 5 ml pipette.
   *Note: the total volume of the cell suspension is now 6 ml.*
7. Take a 20µl sample of the homogenous cell suspension and add to a micro centrifuge tube.
8. Spin down the cell suspension for 3 minutes at 250xg.
9. Aspirate the medium and gently resuspend the cells in 1 ml Pluricyte® Cardiomyocyte Medium.
10. Determine the total cell number and cell viability as follows:

   We highly recommend to perform the cell counting manually using a hemocytometer. For instance, by using the Fuchs Rosenthal Counting Chamber (**Figure 1**):
   
   a. Add 20µl Trypan blue solution to the 20µl cell sample (collected in step 7), mix carefully.
   b. Add 20µl of the Trypan blue/cell suspension mix to the counting chamber.
   c. Calculate the total number of cells according to **equation 1**.

11. Calculate the dilution factor to reach the desired concentration (see **Table 4**) and add PCM to the cell suspension accordingly.
12. Add the cell suspension to a multichannel reservoir using a 5ml pipette.
13. Transfer the coated plate(s) to the flow cabinet and aspirate the excess of the coating solution.
14. Plate cells according to **Table 4**. Make sure to transfer the cells extremely gently.
   *Note: avoid air bubbles and gently resuspend cells in the multichannel reservoir in between pipetting steps to evenly distribute the cells.*
15. Place the plate(s) in the incubator at 37 °C and 5% CO₂.
**Equation 1 Cell counting**

Count 4 #2 squares according to **Figure 1**

Viable cells: ______ + ______ + ______ + ______ = ______ (#vc)

Non-viable (blue) cells: ______ + ______ + ______ + ______ = ______ (#nvc)

$$\frac{\text{#vc}}{4 \times 2 \times 5000} = \frac{\text{total volume after step 6}}{\text{cells/ml}}$$

$$\frac{\text{# of cells/ml}}{\text{cells in total}} = \frac{\text{total volume after step 6}}{\text{total volume after step 6}}$$

Viability = ____: \(\frac{\text{#vc}}{\text{#vc} + \text{nvc}}\) x 100 = _____%

![Figure 1. Lay-out of a Fuchs Rosenthal Counting chamber](image)

**Table 4. Recommended volumes and cell densities per well per plate format**

<table>
<thead>
<tr>
<th>Plate format</th>
<th>Volume to plate per well</th>
<th>Number of cells per well</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well</td>
<td>0.10 ml</td>
<td>25,000-40,000</td>
</tr>
<tr>
<td>48-well</td>
<td>0.25 ml</td>
<td>50,000-100,000</td>
</tr>
<tr>
<td>24-well</td>
<td>0.50 ml</td>
<td>100,000-200,000</td>
</tr>
<tr>
<td>12-well</td>
<td>1.00 ml</td>
<td>200,000-400,000</td>
</tr>
<tr>
<td>6-well</td>
<td>2.00 ml</td>
<td>500,000-800,000</td>
</tr>
<tr>
<td>T25</td>
<td>5.00 ml</td>
<td>1,300,000-1,500,000</td>
</tr>
</tbody>
</table>
3.3 Maintenance of Pluricyte® Cardiomyocytes

It is crucial to always refresh the Pluricyte® Cardiomyocyte Medium (PCM) of the cells one day after seeding the cells (day 1), and subsequently every 2 days (or every 3 days in order to avoid weekend work).

1. Add the required PCM to a 15- or 50 ml tube and warm the PCM to 37°C for 20-30 min. Refer to Table 4 for appropriate volumes.
2. Immediately before use, transfer the warm PCM into a multichannel reservoir and transfer the plate(s) from the incubator to the flow cabinet.
3. Aspirate the PCM from each well.
   *Note: avoid touching the bottom of the wells with the pipette tips to not disturb the cardiomyocyte monolayer.*
4. Add the appropriate volumes pre-warmed PCM per well (Table 4).
5. Transfer the plate(s) back to the incubator.

For further characterization or application of Pluricyte® Cardiomyocytes in different assay platforms, please refer to our application notes for different electrophysiology-based assays. For these assays the recommended assay window is between day 8 and day 12 post-thaw.
4. REFERENCES


2. Pluriomics’ [Pluricyte® Cardiomyocyte product sheet](#).
APPENDIX A. GENERAL TERMS AND CONDITIONS OF SALE

1. Applicability - All Pluriomics’ sales are governed exclusively by the present General Terms and Conditions of Sale as completed by (i) the particular terms agreed in writing between the parties specifying the quantity and type of the items purchased (hereafter the "Products"); and (ii) Pluriomics’ manual of use read and accepted by the buyer (see Pluriomics’ website "http://www.pluriomics.com").

By concluding a sale with Pluriomics, the buyer is conclusively deemed to accept the present General Terms and Conditions of Sale and to waive its general terms and conditions of purchase, of business etc. Parties can only agree on derogations from the General Terms and Conditions of Sale (if any) in writing while acting through duly authorized representatives.

2. Offer - Buyer’s orders are non-binding. A sale shall be concluded only upon written confirmation by Pluriomics of the buyer’s order through a Pluriomics representative with due authority (hereafter the “sale” or the “agreement”). The sale does not include the assignment of intellectual property rights to the buyer, nor does it imply services from Pluriomics to the buyer subsequent to delivery of the Products.

3. Products Warranty - Pluriomics warrants in respect of the Products only that (i) it is the owner of the Products and it is entitled to transfer title of ownership to the buyer (without prejudice to Pluriomics’ and third party intellectual property rights incorporated in the Products and not assigned hereunder); (ii) the Products are manufactured, stored and delivered to the buyer pursuant to article 4 below in compliance with Dutch law; and (iii) the Products meet the technical specifications as set out in Pluriomics’ manual of use and/or the certificate of analysis, both of which are accompanying the Products and are available at Pluriomics’ website http://www.pluriomics.com.

Pluriomics excludes any additional warranties or representations as to the Products in terms of e.g. technical specifications, use, functionality, regulatory compliance, fitness for purpose whether or not disclosed by the buyer, merchantability, etc. By concluding the sale, the buyer conclusively confirms that it received adequate information in order to make a purchase decision, that the Products so purchased meet its requested specifications and that it waives any and all claims against Pluriomics related to the sale except when a breach of the above warranties (i), (ii) or (iii) would be established in accordance with article 10 below (hereafter a "defect"). Defects are either apparent or hidden at the time of delivery.

Pluriomics furthermore makes (i) no warranty or representation as to the validity, scope, or enforceability of the intellectual property rights (including patents licensed hereunder) as further described in article 11 below, and (ii) no warranty or representation that buyer’s exercise of the use license granted in this agreement will not infringe any patents or any intellectual property owned by any third party.

4. Delivery - Delivery to the buyer is made FCA Schiphol (Amsterdam Airport) Netherlands, Incoterms 2010. The buyer shall take delivery at the moment(s) and at the specific point(s) as are communicated by Pluriomics to the buyer. As the case may be, the buyer may be requested to accept partial delivery. Terms of delivery shall always remain indicative and non-binding for Pluriomics. Deliveries performed outside the indicative term of delivery are no ground to refuse the Products, nor shall they justify a claim against Pluriomics for damages, price reduction or otherwise.

The Products will be delivered to the buyer in accordance with the modalities (packaging, temperature, etc.) as set out in the Pluriomics’ manual of use, available at Pluriomics’ website http://www.pluriomics.com.

The buyer will see to it that the subsequent shipment, storing and handling of the Products at its risk and cost will occur in strict compliance with the modalities as set out in Pluriomics’ manual of use, failing which warranty (iii) of article 3 above lapses.

5. Acceptance - Buyer must confirm its non-acceptance of the Products at, or immediately after, delivery as defined in article 4 above. In any case (save for hidden defects), the buyer is conclusively deemed to have accepted the Products five calendar days as from the day of delivery of the Products, unless it sends Pluriomics before the expiry of this period a registered letter stating in a detailed manner the alleged defects (the stamp of the postal services being the relevant time criterion). In case of non-acceptance, the buyer must take all useful measures to allow inspection by Pluriomics and to limit its damage.

6. Title & Risk - Title to the Products and risk of loss or damage to the Products transfers to the buyer as from the moment the Products are placed at the disposal of the buyer or the buyer’s representative pursuant to article 4 above.

7. Price - Prices are stated and payable in Euro. Prices include shipment till the point of delivery, packaging of the Products, the cost of customs formalities for export, as well as all duties, taxes and other charges payable upon export (however excluding e.g. inspection costs, as well as all duties, taxes and other charges related to the import of the Products and their transit through any third country).
8. Payment - Invoices are irrevocably deemed accepted by the buyer failing protest by registered letter within thirty (30) calendar days. Invoices for the sale of Products are due on the date as stated therein. Invoices shall be settled on Pluriomics’ bank account as a cleared payment on the due date indicated in the invoice, all settlement costs remaining with the buyer. Settlement of all Pluriomics’ invoices is required as a condition to initiate shipment to the point of delivery as set out in article 4 above.

In case Pluriomics does not receive payment on the due date, late interest will be due automatically from the due date. The rate of this late interest shall be equal to the Dutch statutory commercial interest rate or 1 % per month started, whichever is the highest. In addition, in order to compensate Pluriomics’ additional damage, such as administrative and other costs (excluding legal fees), resulting from the non-payment on the due date, the buyer shall owe liquidated damages equalling 10% of the amount of the unpaid invoice with a minimum of 250,00 Euro, due as from the due date.

Without prejudice to the foregoing and to any other right Pluriomics may have by law, if an invoice is not paid on its due date, Pluriomics is entitled without any formality (such as a prior notice of default or a court intervention) to claim immediate payment of all amounts owed to Pluriomics by the buyer, even if not yet payable, and to suspend or cancel the relevant order or any other outstanding order of the buyer by giving written notice thereof to the buyer. In case Pluriomics opts for cancelling the relevant order and/or any of the outstanding orders of the buyer, the latter will owe Pluriomics, as a lump sum compensation for not executing the order(s), an amount equal to 30 % of the agreed price of the cancelled order(s).

9. Restrictions of use of the Products and hold harmless - The buyer will use the Products acquired for internal research purposes only, subject to the restrictions of use set out herein, solely benefiting the buyer. No other right is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of the Products does not include nor carry any right or license to use, develop or otherwise exploit the Products commercially, and no rights are conveyed to the buyer to use the Products for any other purpose than internal research.

The buyer agrees to use the Products in compliance with all applicable statutes and regulations, but not to use the Products for research involving administration and/or use of Products for human or animal therapeutic, diagnostic and/or prophylactic purposes including but not limited to clinical applications, cell therapy, transplantation, and/or regenerative medicine (whether or not such administration and/or use would be authorized under applicable law).

The buyer agrees not to sell, donate or otherwise transfer the Products to a third party, nor to place the Products under the custody of a third party. Furthermore, the buyer agrees to apply to the induced Pluripotent Stem Cell derived Pluricyte® Cardiomycocytes the Pluricyte® culture medium, excluding any other medium or product originated from the buyer and/or a third party.

The restrictions set forth herein apply to the Products, their components and the derivatives of the Products and their components.

The buyer shall defend, indemnify and hold harmless Pluriomics, IPS Academia Japan Inc. and Kyoto University, their affiliated undertakings, the inventors of the licensed patents and their directors, officers, employees and agents (together, the “Indemnified Entities”) from and against any and all claims, liabilities, losses, damages or expenses (including but not limited to reasonable attorneys’ fees and other costs of defending any action) that any of the Indemnified Entities may sustain or incur as a result of any claim of a third party based on the negligence, recklessness or willful misconduct of the buyer or any of its employees or agents in performing its obligations or exercising the rights, including the license to use, as granted pursuant to this agreement.

10. Defects - Products which suffer from a defect, as recognized by Pluriomics or as held by court, shall, at Pluriomics’ discretion, either be replaced free of charge or shall be reimbursed in part or in full. Pluriomics shall not owe the buyer any indemnity unless the buyer proves that Pluriomics has committed gross negligence.

In any case where an indemnity would be due, such indemnity will cover buyer’s direct damage only and will not cover indirect damage such as, without limitation, loss of production, turnover or profit, loss of business opportunities, loss of data, loss of time or reputational damage, even if Pluriomics has been advised up front of the possibility of such losses or damage.

Hidden defects must be notified by registered letter to Pluriomics within seven calendar days from their discovery, failing which the buyer’s claim for such defects will not be admissible.

Any legal claim against Pluriomics must be filed in court no later than 18 months as of the moment the Products are placed at the disposal of the buyer or the buyer’s representative pursuant to article 4 above, failing which such claim is inadmissible.
11. Licensing and sublicensing - The Products sold hereunder include intellectual property rights developed by Pluriomics and (sub)licensed by Pluriomics from a third party. Such intellectual property rights are protected by the laws on patents, copyrights, trade secrets, and other laws.

Pluriomics has filed per June 10, 2013 for a patent relating to differentiation and culturing of stem cell derived cardiomyocytes.

Details about the intellectual property rights developed by Pluriomics and (sub) licensed by Pluriomics from a third party are set out in the manual of use accompanying the Products and also available at Pluriomics' website "http://www.pluriomics.com".

The buyer acknowledges and agrees that the present sale does not transfer to the buyer any title in respect of the intellectual property rights incorporated in the Products, nor does it grant any other intellectual property rights except a limited non-transferable and non-exclusive license to use the Products in accordance with the use restrictions set out in article 9 above.

The buyer hereby grants to Pluriomics, IPS Academia Japan Inc. and Kyoto University a non-exclusive, worldwide, perpetual, royalty-free, fully paid up license to use for any purpose any and all improvements made by the buyer to the Products covered by buyer’s Improvement Patents. For the purpose of this provision, “buyer’s Improvement Patents” means any patent or any patent application owned by the buyer that covers any improvements made by the buyer in course of or as a result of the exercise by the buyer of the right granted by Pluriomics under this agreement. In addition, the buyer hereby grants to IPS Academia Japan Inc. a license right with sublicense right under the buyer’s Improvement Patents so that IPS Academia Japan Inc. may grant royalty-free licenses under the buyer’s Improvement Patents to Kyoto University and other bona fide non-commercial academic institutions in Japan for academic research and educational purposes.

Pending the issuance of a patent, the buyer shall mark the objects so made (or their containers or labels) with the words "Patent Pending," and following the issuance of one or more patents, with the numbers of any applicable patent(s).

12. Patent enforcement - Pluriomics, IPS Academia Japan Inc. or Kyoto University (depending on who is the owner of the relevant patent) shall have, at its expense, the sole right to file for, prosecute and maintain the patents licensed hereunder, using patent counsel of its choice. It is the present intention of Pluriomics, IPS Academia Japan Inc. or Kyoto University, as the case may be, to pursue prosecution of applications of, and maintain, the patents licensed hereunder. However, the buyer acknowledges that neither Pluriomics nor IPS Academia Japan Inc., nor Kyoto University shall, in any event, be responsible to the buyer for any act or omission relating to the preparation, prosecution and maintenance of any and all patent applications or patents.

Pluriomics, IPS Academia Japan Inc. or Kyoto University (depending on who is the owner of the relevant patent) intend to enforce their patents against infringers or otherwise act to eliminate infringement, when, in such owner’s sole judgment, such action may be reasonably necessary, proper, and justified and makes reasonable business sense considering all factors. In the event that the buyer believes there is infringement of a patent under this agreement which is to the buyer’s substantial detriment, the buyer may, subject to any of its obligations of confidentiality, provide Pluriomics with notification and reasonable evidence of such infringement. If action is taken by the owner to remedy the infringement, the buyer shall provide commercially reasonable assistance as requested by the owner. Nonetheless, Pluriomics, IPS Academia Japan Inc. or Kyoto University (depending on who is the owner of the relevant patent) is under no obligation to bring any action or proceeding against any entity for infringing of the patent.

13. Force majeure - Action by government, change in legislation or policy, war, riots, strikes, lock-outs, fire, breakdown of machines, inadequate supply of materials or energy, interruption in transport or any other circumstances beyond Pluriomics’ control, which hinder the normal performance of Pluriomics’ obligations hereunder, shall be considered by the parties to constitute force majeure involving suspension or termination of the agreement, at Pluriomics’ option and at no cost for either party.

14. Unenforceability - In the event all or part of a provision hereof were to be declared unenforceable, the enforceable part of said provision and all other provisions hereof will remain valid and effective while the unenforceable provision or part thereof will be replaced by an enforceable one, having a similar effect. Parties confirm that the sanctions and liquidated damages set out in these General Terms and Conditions of Sale are reasonable and proportionate in light of the anticipated loss likely suffered by the aggrieved party.

15. Applicable law & competent court - Dutch law, excluding the 1980 Convention on International Sale of Products, is applicable to this agreement. Any disputes relating to the existence, the interpretation, the performance or the termination of this agreement shall be submitted to the exclusive competence of the Courts of Amsterdam (the Netherlands), it being understood that Pluriomics reserves the right to bring any dispute before the courts of the buyer’s place of residence.