

## Human Cardiac Myocytes

Cardiac muscle, a unique striated muscle in the human heart, is composed of single Cardiac Myocytes. Because they are responsible for the myogenic contraction of the whole cardiac muscle, Cardiac Myocytes are the most physically energetic cells in the body.

PromoCell Human Cardiac Myocytes (HCM) are isolated at PromoCell's cell culture facility from normal human ventricle tissue of the adult heart. Shortly after isolation, all PromoCell HCM are cryopreserved at passage 2 (P2). Therefore the HCM have a high potency for proliferating after thawing.

PromoCell HCM are ideal for *in vitro* research on cardiac diseases like hypertrophy and for physiological and pharmacological studies. Unlike freshly isolated "rod-shaped" myocytes, PromoCell HCM are suitable for long-term experiments such as investigating the long-term effects of cytokines, mechanical strains, or cell-cell interactions.

### Features of PromoCell HCM

- Adult origin
- Proliferating cells
- Cardiac myocyte marker positive
- Qualified for long-term experiments
- Cryopreserved at second passage

### Morphology of HCM in culture

In culture HCM show two distinct morphologies depending on the density of the cells. When the cells are cultured at low density, the HCM grow flat and display short cellular extensions (Fig. 1a). With increasing cell density a second cell morphology is visible. The cells appear thin, elongated and grow on top of each other in clusters (Fig. 1b). Between these clusters, flat cells are arranged in parallel.

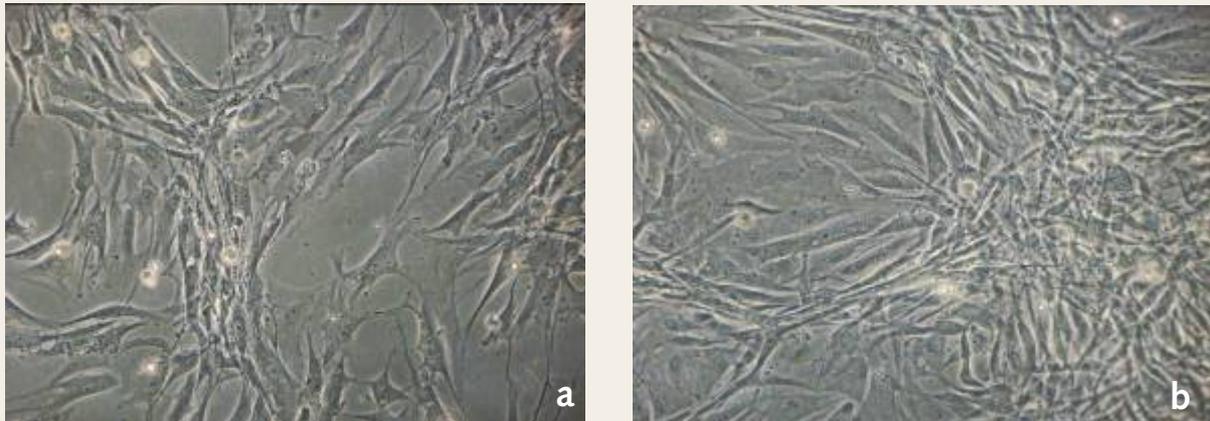


Fig. 1: The distinct morphologies of HCM. In low density culture the HCM are flat and have short cellular extensions (a). With higher density a second cell morphology is visible. These cells appear thin, elongated and grow on top of each other in clusters (b).

## Myocyte Growth Medium

PromoCell has engineered a specialised medium for the growth and proliferation of HCM. The PromoCell Myocyte Growth Medium is a low serum (5% V/V) medium, supplemented with optimized hormones and growth factors.

Final supplement concentrations  
(after addition to the medium)

■ Fetal Calf Serum	0.05 ml / ml
■ Epidermal Growth Factor (recombinant human)	0.5 ng / ml
■ Basic Fibroblast Growth Factor (recombinant human)	2 ng / ml
■ Insulin (recombinant human)	5 µg / ml

## Quality Control

Rigid quality control tests are performed for each lot of PromoCell Human Cardiac Myocytes. HCM are tested for cell morphology, adherence rate, and cell viability. In addition, Cardiac Myocyte specific markers are determined by flow cytometric analysis. Growth performance is also tested through multiple passages up to 15 population doublings (PD).

### Quality Control of PromoCell HCM

#### Viability

- Determination of viable cells
- > 500.000 viable cells in each cryovial

#### Growth performance

- Determination of Population Doubling time in the log-phase
- Testing that cells reach > 15 Population Doubling in culture

#### Phenotypic Characterization

- Detection of cell-type specific markers by flow cytometry

## Order Information

PromoCell HCM can be ordered as either cryopreserved cells or as proliferating cells. If the customer orders cryopreserved cells, he/she receives a cryovial which contains more than 500,000 viable cells after thawing. Cells are shipped on dry ice and should be stored in liquid nitrogen or cultured immediately upon arrival.

When proliferating cells are ordered, the cells are seeded (500,000 cells) at PromoCell and cultured for three days before shipping. They are transported with warm packs and shipped on Mondays. Upon arrival the customer can work with the cells immediately.

PromoCell delivery time is 24-48 h door to door.

Product	Size	Catalog Number
Human Cardiac Myocytes (HCM)	500,000 cryopreserved cells	C-12810
	500,000 proliferating cells	C-12811

## PromoCell Ethical Standards

PromoCell is the original manufacturer of Human Cardiac Myocytes and is committed to the highest ethical standards. Consequently, PromoCell strictly complies with the following procedure for the donation and collection of human tissue used for cell isolation.

The tissue used by PromoCell for the isolation of human cell cultures is derived from donors who have signed an informed consent form (this being done by the donor himself, an authorized agent, or a legal agent) which outlines in detail the purpose of the donation and the procedure for processing tissue.

Because of these high ethical standards PromoCell is certified by GlaxoSmithKline as approved Global Supplier.

## Cultivation of HCM

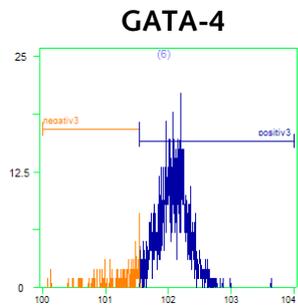
The morphology of PromoCell Human Cardiac Myocytes changes over the course of long-term cultivation. We have evaluated HCM cell-specific markers at several time points throughout this culture period.

Human Cardiac Myocytes express distinct markers at each stage of differentiation. The earliest markers are GATA-4 and  $\alpha$ -Sarcomeric Actin. In late phase differentiation structural proteins are produced, namely  $\alpha$ -Sarcomeric Actinin and Slow Myosin Heavy Chain. Terminal differentiation is characterized by the accumulation of sarcomeric structures, of which Troponin T-C and Myosin regulator light chain 2 (MYL2) are key proteins.

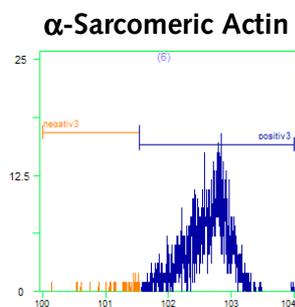
We have analyzed the presence of all 6 markers of HCM differentiation after 21, 42, and 60 days of cultivation.

## 21 days cultivation

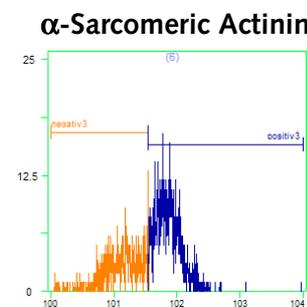
### Cell-specific markers of HCM differentiation as analyzed by Flow Cytometry



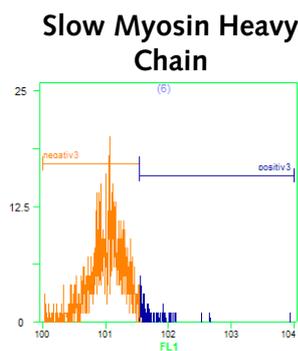
92% of the cells are GATA-4 positive.



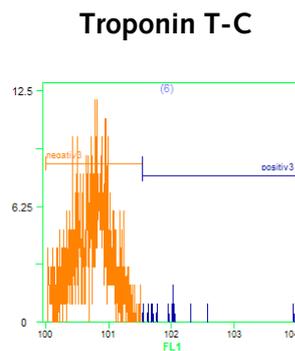
98% of the cells are  $\alpha$ -Sarcomeric Actin positive.



64% of the cells are  $\alpha$ -Sarcomeric Actinin positive.



4% of the cells are Slow Myosin Heavy Chain positive.



1% of the cells are Troponin T-C positive.

## Morphology after 21 days

HCM are thin and elongated. They grow on top of each other.



HCM in phase contrast after 21 d cultivation.

## Results after 21 days

Over 90% of the HCM are positive for markers of early differentiation. Between 4 and 64% are positive for markers of late differentiation. The HCM are negative for markers of terminal differentiation.

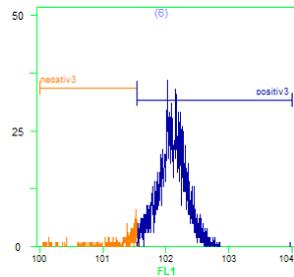
GATA-4	92%
$\alpha$ -Sarcomeric Actin	98%
$\alpha$ -Sarcomeric Actinin	64%
Slow Myosin Heavy Chain	4%
Troponin T-C	1%
MYL-2	-

⇒ The HCM are in an early stage of differentiation after 21 days of cultivation.

## 42 days cultivation

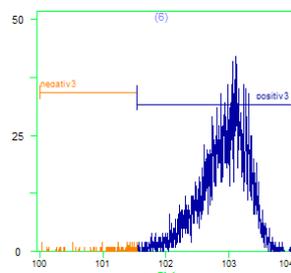
### Cell-specific markers of HCM differentiation as analyzed by Flow Cytometry

#### GATA-4



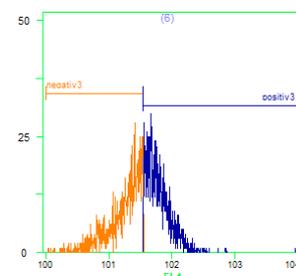
95% of the cells are GATA-4 positive.

#### $\alpha$ -Sarcomeric Actin



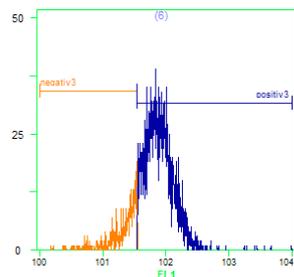
99% of the cells are  $\alpha$ -Sarcomeric Actin positive.

#### $\alpha$ -Sarcomeric Actinin



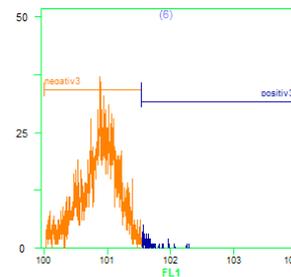
49% of the cells are  $\alpha$ -Sarcomeric Actinin positive.

#### Slow Myosin Heavy Chain



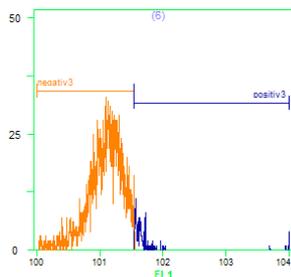
85% of the cells are Slow Myosin Heavy Chain positive.

#### Troponin T-C



1,5% of the cells are Troponin T-C positive.

#### MYL2



5% of the cells are MYL2 positive.

## Morphology after 42 days

HCM begin to form myotube-like structures.



HCM in phase contrast after 42 d cultivation. HCM begin to form myotube-like structures (arrow)

## Results after 42 days

Over 95% of the HCM are positive for markers of early differentiation. Between 49 and 85% are positive for markers of late differentiation. Approximately 5% of the HCM are positive for markers of terminal differentiation. The HCM are now in late stage differentiation.

GATA-4	95%
$\alpha$ -Sarcomeric Actin	99%
$\alpha$ -Sarcomeric Actinin	49%
Slow Myosin Heavy Chain	85%
Troponin T-C	1,5%
MYL-2	5%

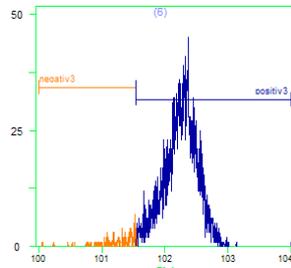
The morphology of the cells supports the above result that the HCM are undergoing late differentiation. The myotube-like structures are typical for differentiated Cardiac myocytes.

⇒ There is a significant increase of HCM positive for the markers of late differentiation. At the same time the HCM show typical morphological features of Cardiac Myocytes in late differentiation. The HCM are in a late stage of differentiation after 42 days cultivation.

## 60 days cultivation

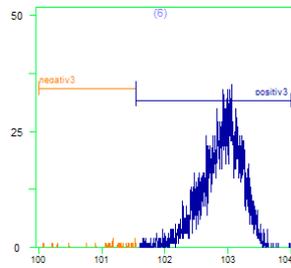
### Cell-specific markers of HCM differentiation as analyzed by Flow Cytometry

#### GATA-4



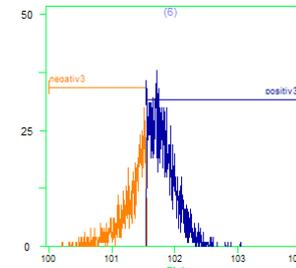
96% of the cells are GATA-4 positive.

#### $\alpha$ -Sarcomeric Actin



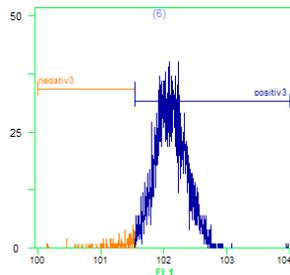
99% of the cells are  $\alpha$ -Sarcomeric Actin positive.

#### $\alpha$ -Sarcomeric Actinin



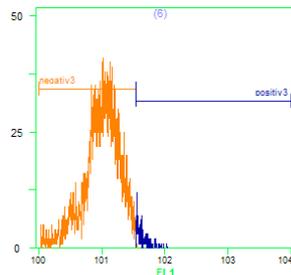
66% of the cells are  $\alpha$ -Sarcomeric Actinin positive.

#### Slow Myosin Heavy Chain



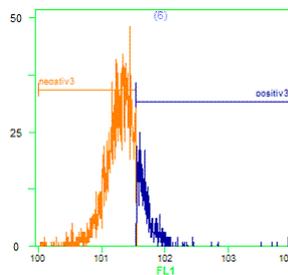
98% of the cells are Slow Myosin Heavy Chain positive.

#### Troponin T-C



3% of the cells are Troponin T-C positive.

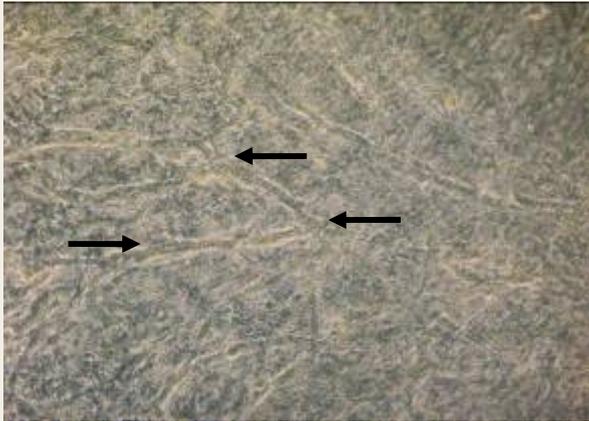
#### MYL-2



19% of the cells are MYL2 positive.

## Morphology after 60 days

The number of HCM with myotube-like structures increases. The HCM with myotube-like structures begin to form branch-like structures.



HCM in phase contrast after 60 d cultivation. The number of myotubes increases and the myotubes branch (arrows).

## Results

Over 95% of the HCM are positive for markers of early stage differentiation. Between 66 and 98% are positive for markers of late differentiation. Approximately 3 to 19% of the HCM are positive for markers of terminal differentiation. The HCM are now in late stage differentiation and 20% have reached the terminal stage of differentiation.

GATA-4	96%
$\alpha$ -Sarcomeric Actin	99%
$\alpha$ -Sarcomeric Actinin	66%
Slow Myosin Heavy Chain	98%
Troponin T-C	3%
MYL-2	19%

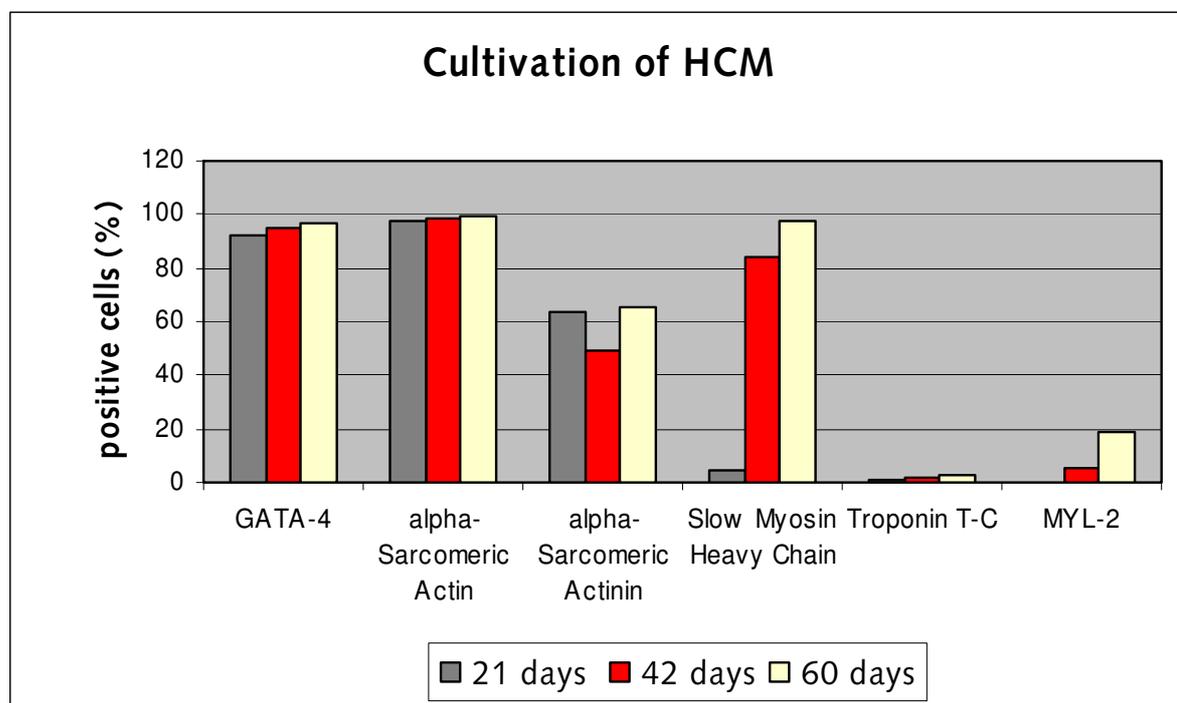
These results are also supported by the morphology of the HCM. The number of cells that form myotube-like structures increases and the cells form branch-like structures.

These branching structures are typical for terminal differentiation of Cardiac Myocytes. Skeletal myotubes in comparison, do not form these branch-like structures.

⇒ There is an increase of HCM positive for the markers of terminal differentiation. At the same time the HCM show branch-like structures, a typical morphological feature of Cardiac Myocytes in terminal differentiation.

## Summary

PromoCell HCM proliferate in culture and have the ability to reach more than 15 population doublings. During this growth phase, PromoCell HCM remain in an early differentiation stage.



During long-term cultivation of PromoCell HCM, the expression of markers of differentiation change. With longer culture time HCM express positive markers of late differentiation and up to 20% of HCM express positive markers of terminal differentiation.

When grown to confluency, PromoCell HCM begin to differentiate and over time reach the terminal stage of differentiation. Differentiation can be seen by morphological alterations in long-term culture and by the appearance of stage specific markers of differentiation.

The morphology of PromoCell HCM changes over time. Myotube-like structures, typical for differentiated Cardiac Myocytes are initially detected in culture. Thereafter, when the cells are let to grow continually, the myotubes start to form branch-like structures. These branch-like structures are typical for terminal differentiated Cardiac Myocytes.

Results are supported by the analysis of markers of differentiation at different time points during PromoCell HCM culture. Where the myotube-like structures are detected, an increase in markers of late differentiation is observed. With longer culture time, the numbers of HCM that show the markers of late differentiation increases and up to 20% of the HCM express markers of the terminal differentiation. At these time points PromoCell HCM start to form branching structures.

### Features of PromoCell HCM in culture

- Proliferating HCM are in an early stage of differentiation.
- HCM can reach over 15 population doublings. They have a high capacity for proliferation.
- When the HCM are grown to confluency, the differentiation process begins.
- The HCM form myotube-like structures and branch-like structures.
- In culture the HCM can reach the terminal phase of differentiation.

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