

# Cell Culture

LIVE

Volume 8



**PAA**  
THE CELL CULTURE COMPANY

## CryoMaxx NT

DMSO free cryopreservation

Neuronal  
Base Medium P  
Affixene  
siRNA Prime Kit  
EndoPrime Kit  
HepatoPrime Kit  
Hybridoma Pro  
JuLI™



## News from PAA

### Discover Our New Products

Cell culture is all about growth, which is also true for PAA as one of the world leading cell culture companies. Over the last few months, our portfolio of innovative cell culture products has expanded significantly to include media for cryopreservation, neural cell culture, endothelial cell media and more. You can discover some of these new products in our latest issue of "Cell Culture Live".

### Animal Sera

Besides Fetal Bovine Serum from diverse origins, PAA also offers a wide range of other animal sera. For special applications such as autologous cell culture systems, immune histochemistry and immunoblotting, PAA's portfolio of animal sera includes Horse, Porcine, Sheep, Rabbit, Goat, Rat, Mouse and Chicken Serum.

If you require any other type of animal sera from the aforementioned, then please contact your local PAA representative or our customer services.

### Customized Manufacturing

The manufacturing of customized products is a speciality of PAA. Tailor-made products, customized fillings and special packaging are part of our superb portfolio.

PAA can offer customized liquid media in batch sizes from 10 to 2500 L and specially prepared powder media in batch sizes from 0.5 to 8000 kg, manufactured under pharmaceutical GMP conditions in our state-of-the-art production facility in Pasching, Austria.

The lead time for liquid media, inclusive of production and all QC/QA testing is 8 weeks maximum; the lead time for powder production is 4 weeks. Extended tests can be carried out upon request.

Our customized liquid media can be filled into PET bottles, Flexboy Bags, Cubitainers, Biotainers or other types of packaging. Powder media can be packed in our standard plastic containers up to 950 ml. Other types of packaging including bulk packaging are available on request.

Enjoy the eighth issue of "Cell Culture Live"



Harry Brack  
(Chief Operating Officer)



# CryoMaxx NT

## DMSO free Cryopreservation with Highest Cell Viability

Cryopreservation is a procedure to stabilize cells at cryogenic temperatures for long-term storage. Cells contain fragile membranes and organelles which can be destroyed by ice formation during the freezing procedure. The uses of cryoprotective additives or chemicals protect the cells during the freezing process by minimizing the damage associated with ice crystal formation.

DMSO is most commonly used for cryoconservation because of its ability to reduce ice crystal formation. However, for certain applications like re-implantation experiments, it is more beneficial to work without DMSO.



### CryoMaxx NT – for Special Demands

PAA has developed CryoMaxx NT (DMSO free and serum-free) freezing media to accommodate DMSO sensitive cells and specific applications. The viability of cells cultured in DMSO free freezing media is generally much lower when compared to DMSO containing media. However, our research has shown that CryoMaxx NT produces superior cell viability (more than double) in comparison to other DMSO free cryopreservation products.

#### Order Information

|             |         |       |
|-------------|---------|-------|
| CryoMaxx NT | J05-019 | 50 ml |
|-------------|---------|-------|



#### Applications

- ▶ Cryopreservation of DMSO sensitive cells
- ▶ Cryopreservation of cell lines and primary cells
- ▶ Re-implantation experiments in animals
- ▶ Cell banking

#### Features

- ▶ Serum-free and DMSO free
- ▶ The best cell viability recovery level of any DMSO free product
- ▶ Ready to use medium
- ▶ Simple freezing protocol

# Neuronal Base Medium P

## The Ideal Basal Medium for Individual Applications

Detailed studies of neurons and neural stem cells require carefully defined culture conditions. Nutrient media supplemented with fetal bovine serum contain factors which can inhibit the growth of neuronal cells. Therefore, PAA has developed specific serum-free media optimized for the survival and growth of both embryonic and adult neurons.

PAA's Neuronal Base Medium P is a new media formulation which meets the special requirements of adult and postnatal neurons and provides an optimal environment for the proliferation and differentiation of neural stem cells and neurospheres. This medium can be used in combination with different supplements (NeuroMix, Neuronal Stem Cell Supplement) to facilitate the growth of adult neuronal cells without the need of an astrocyte feeder layer.

### Applications

- ▶ Expansion of neural stem cells and neurospheres (with Neuronal Stem Cell Supplement and G5)
- ▶ Differentiation of neural stem cells (with NeuroMix and G5)
- ▶ Growth of oligodendrocytes and astrocytes (with NeuroMix)
- ▶ Culturing of adult neurons, neural stem cells and neurospheres (with NeuroMix)

### Features

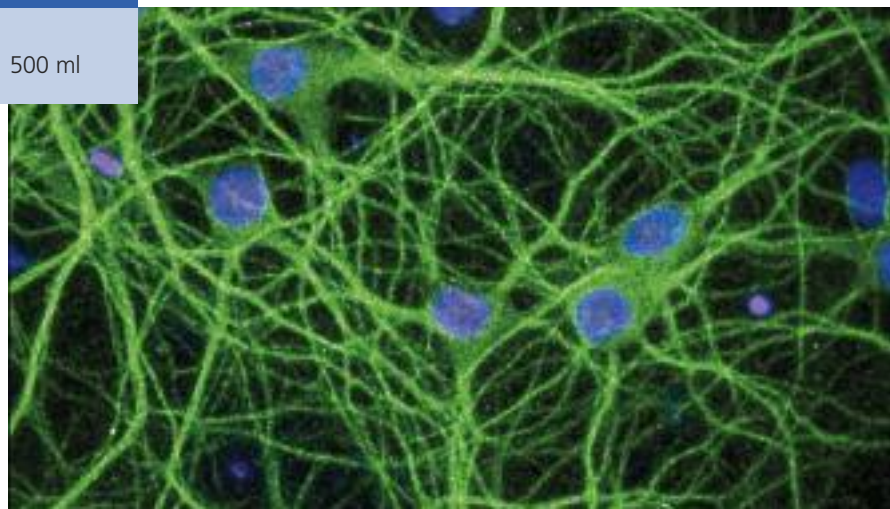
- ▶ Long-term growth of postnatal and adult neurons
- ▶ Successful expansion and differentiation of neural stem cell and neurospheres
- ▶ No feeder cells necessary
- ▶ Serum-free

### Order Information

Neuronal Base Medium P,  
without L-Glutamine

U15-059

500 ml



# Affixene

## Optimal Attachment Factor for Neural Cells

Growth and development of many cells depend on specific attachment factors and extracellular matrix components. Cell culture vessels are often coated with different attachment factors to enhance the proliferation and differentiation of cells. Most attachment factors are unfortunately derived from animal origins which could introduce adventitious agents into the cell culture system.

PAA has developed Affixene, a synthetic and chemically defined attachment factor tailored to meet the specific requirements of neural cells. Affixene improves plating efficiency which enhances cell spreading and proliferation.

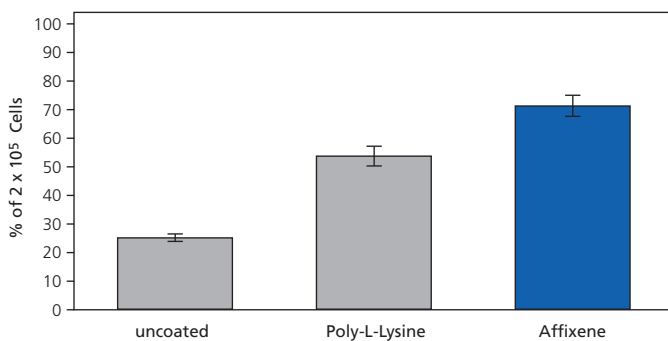
### Applications

- ▶ Attachment factor for neural cells
- ▶ Coating of cell culture vessels
- ▶ Suitable for serum reduced and serum-free applications

### Features

- ▶ Improved plating efficiency
- ▶ Increased stability of coating
- ▶ Easy to handle
- ▶ Chemically defined
- ▶ Animal component free (ACF)
- ▶ No batch-to-batch variations

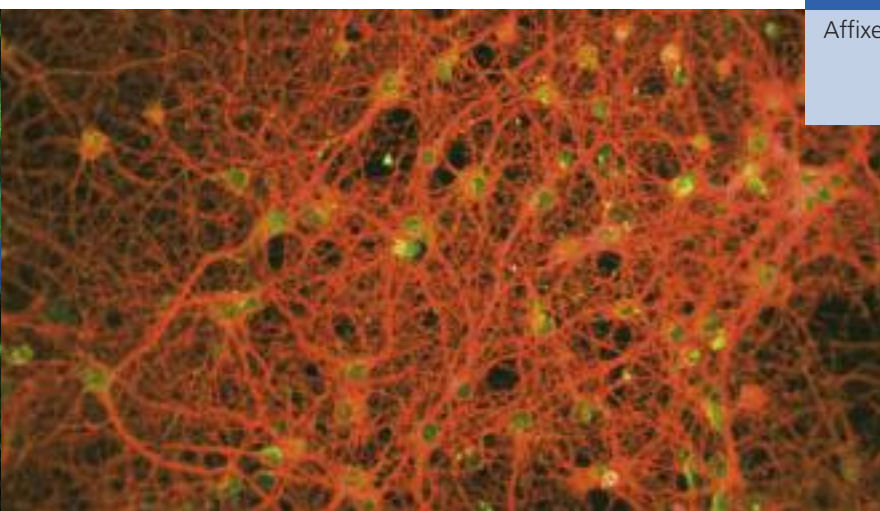
Cell Adhesion



Primary hippocampal neurons were plated onto Poly-L-Lysine and Affixene coated 24 well plates at a density of  $2 \times 10^5$  cells per well.

### Order Information

|          |         |       |
|----------|---------|-------|
| Affixene | F01-042 | 10 ml |
|          | F05-042 | 50 ml |

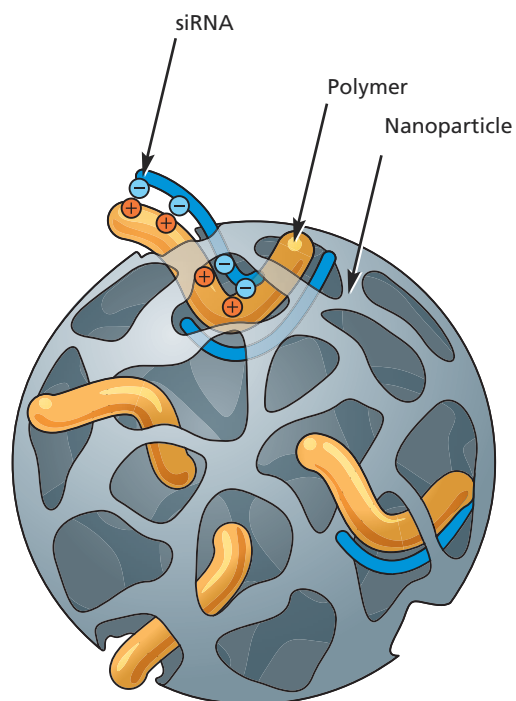


# siRNA Prime Kit

## Efficient Gene Silencing & Gene Knockout

PAA's siRNA Prime Kit contains an excellent transfection reagent and special buffer that provides efficient siRNA silencing and gene knockout of more than 90%. Our unique transfection reagent uses nanotechnology in combination with the specially developed siRNA Prime Buffer to provide a stable and consistent environment, and therefore high transfection efficiency. Importantly, the nanoparticles have a highly protective structure and the binding of the siRNA to positively charged carrier molecules enhances the incorporation of the siRNA in the cells.

PAA's siRNA Prime Kit allows transfection of siRNA and miRNA; it is suitable for a broad range of cell lines and primary cells. The time saving and easy protocol eliminates the need for laborious siRNA optimization.



The unique structure of a nanoparticle protects the siRNA against degradation from endonucleases.

### Applications

- ▶ Transfection of siRNA and miRNA
- ▶ Gene silencing
- ▶ Gene expression analysis
- ▶ High throughput screening
- ▶ Suitable for many cell lines

### Features

- ▶ Silencing with minimal siRNA quantities
- ▶ Efficient gene knockout
- ▶ Excellent cell viability
- ▶ Transfection in presence or absence of serum
- ▶ Time saving and easy protocol

### Order Information

|                 |          |        |
|-----------------|----------|--------|
| siRNA Prime Kit | Q050-042 | 0.2 ml |
|                 | Q051-042 | 1 ml   |

# EndoPrime Kit

## Flexible Kit System for Optimal Growth of Endothelial Cells

The cultivation of primary endothelial cells as well as their proliferation require special cell culture conditions. The EndoPrime Kit is a flexible system for micro- and macrovascular endothelial cells. The advanced formulation supports the growth of primary human and animal cells in a low-serum environment. Extensive experimental testing has proven that EndoPrime facilitates both excellent cell morphology and proliferation rates.

Certain applications or experiments may require full control over the media formulation, in particular the specific concentrations of important growth factors such as EGF and VEGF. Therefore, PAA's modular kit system provides you with the flexibility to optimize the medium to your specific needs.

### Features

- ▶ Convenient kit system for individual optimization
- ▶ Best viability
- ▶ Highest proliferation rate
- ▶ Excellent cell morphology
- ▶ Xeno-free application with EndoPrime HS

### Suitable for:

#### Microvascular Cells

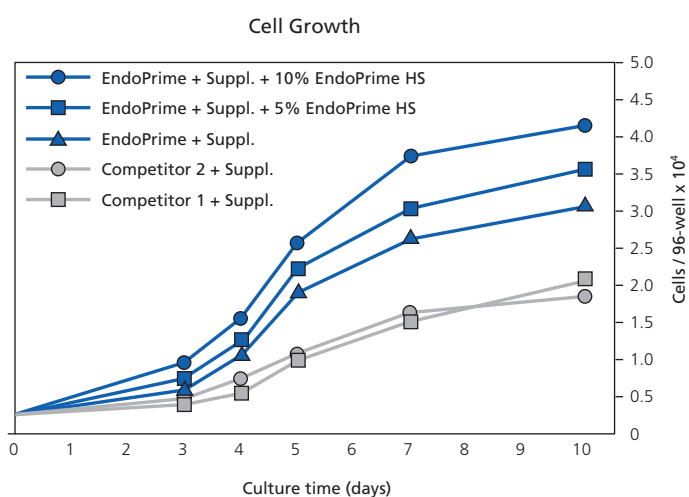
HDMEC (Human Dermal Microvascular Endothelial Cells)  
 HDLEC (Human Dermal Lymphatic Endothelial Cells)  
 HBdMEC (Human Bladder Microvascular Endothelial Cells)  
 HCMEC (Human Cardiac Microvascular Endothelial Cells)  
 HPMEC (Human Pulmonary Microvascular Endothelial Cells)  
 HUtMEC (Human Uterine Microvascular Endothelial Cells)

#### Macrovascular Cells

HUVEC (Human Umbilical Vein Endothelial Cells)  
 HUAEC (Human Umbilical Artery Endothelial Cells)  
 HPAEC (Human Pulmonary Artery Endothelial Cells)  
 HSVEC (Human Saphenous Vein Endothelial cells)  
 HAoEC (Human Aortic Endothelial Cells)  
 HCAEC (Human Coronary Artery Endothelial Cells)

### EndoPrime HS – as a Little Extra

To improve the growth characteristics even further, a special human serum (Endo Prime HS) can be added to the Endo Prime Medium. For xeno-free applications, EndoPrime FBS can even be completely substituted by EndoPrime HS.



Growth kinetics of HSVEC cells cultured in different complete media. EndoPrime Kit supplemented with 5 and 10% EndoPrime HS showed a significant increase in growth compared to competitors' media.

### Order Information

|               |          |       |
|---------------|----------|-------|
| EndoPrime Kit | U050-042 | 1 Kit |
| EndoPrime HS  | C025-029 | 25 ml |

### EndoPrime Kit contains

- ▶ EndoPrime Base Medium 500 ml
- ▶ EndoPrime Supplement Mix (100x) 5 ml
- ▶ EndoPrime EGF (2.5 µg/ml) 1 ml
- ▶ EndoPrime VEGF (250 ng/ml) 1 ml
- ▶ EndoPrime FBS 25 ml

# HepatoPrime Kit

## Innovative Hepatocyte Media for Reliable Results

Hepatocytes that have been isolated from the liver play an important role in the processing of endogenous and exogenous substances. It is well documented that primary cultures of human and rodent hepatocytes can be used to examine drug-drug interactions, hepatotoxicity, transporter activity and are routinely used for important studies on metabolic processes including cytochrome P450 activity assays. The aforementioned *in vitro* model is suitable for experiments fulfilling the requirements of REACH (Registration, Evaluation and Authorization of CHemicals), the regulatory initiative of the EU Commission for the safety of chemicals.

### HepatoPrime ST Kit – ideal maintenance medium for short-term experiments

Primary hepatocytes normally have a limited life expectancy in culture making it difficult for experimental analyses. The new HepatoPrime ST Kit has been developed as an efficient maintenance medium for short-term studies and basic research. Displaying the typical hepatocyte features, reliable results in studies of drug metabolism, pharmacology and toxicity will be ensured (e.g. improved inducibility of the CYP450 system). The HepatoPrime ST base medium is a modified William's Medium E; the corresponding supplement includes ITS, dexamethasone and other growth factors.

### HepatoPrime LT Kit – for long-term cultivation of hepatocytes

The new HepatoPrime LT Kit has been developed for long-term cultivation of hepatocytes, extending life expectancy and functionality of these cells. This rich medium enables the cultivation of hepatocytes for about four weeks, thus allowing for extended studies on drug metabolism, pharmacology, toxicology and basic research. The HepatoPrime LT base medium is a modified version of William's Medium E; the supplement provided in the kit contains ITS, hydrocortisone, EGF and other growth additives.

### Applications

- ▶ Induction and inhibition assays
- ▶ Transporter and acute toxicity studies
- ▶ Subchronic toxicity studies
- ▶ Drug pre-screening
- ▶ Liver cell regulation and regeneration
- ▶ Analyses of active substances according to REACH

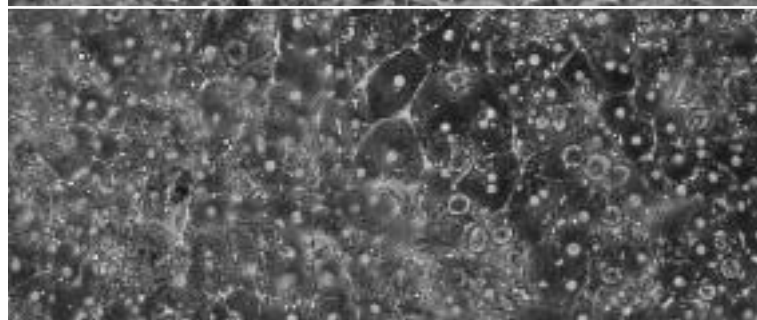
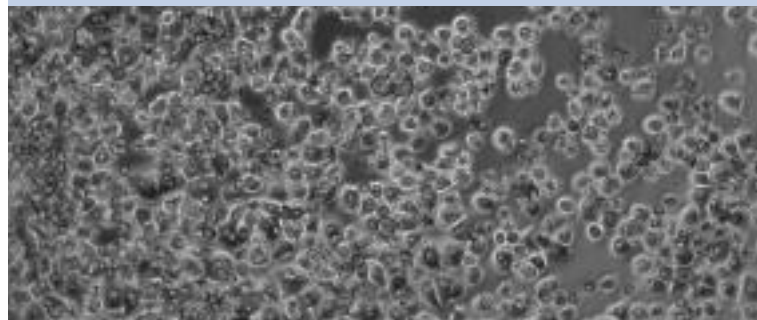
### Features

- ▶ Highest viability
- ▶ Maintenance of metabolic activity
- ▶ Highest activity of cytochrome P450 system

Primary hepatocytes cultivated in HepatoPrime ST:  
Above on the day of seeding; below after 7 days.

### Order Information

|                            |          |          |
|----------------------------|----------|----------|
| <b>HepatoPrime ST Kit</b>  | U050-075 | U051-075 |
| HepatoPrime ST Base Medium | 100 ml   | 500 ml   |
| HepatoPrime ST Supplement  | 1 ml     | 5 x 1 ml |
| <b>HepatoPrime LT Kit</b>  | U050-073 | U051-073 |
| HepatoPrime LT Base Medium | 100 ml   | 500 ml   |
| HepatoPrime LT Supplement  | 1 ml     | 5 x 1 ml |



# Hybridoma Pro

## The Optimal Production Medium for Highest Antibody Yield

Hybridoma cells are used widely in cell culture to produce large quantities of monoclonal antibodies. With Hybridoma Pro, PAA's newly developed serum-free medium, hybridoma cells will produce high antibody titres. The medium formulation is perfectly optimised to provide high cell density growth and optimal conditions for maximum antibody production. In addition, the medium has been extensively tested and shown to reduce the shear forces on the cells and thus high cell viability is always achieved. Moreover, the low protein content of the medium makes the downstream purification of antibodies much simpler. Hybridoma Pro can be used with all hybridoma cell lines or NS0 myeloma cells.

### Applications

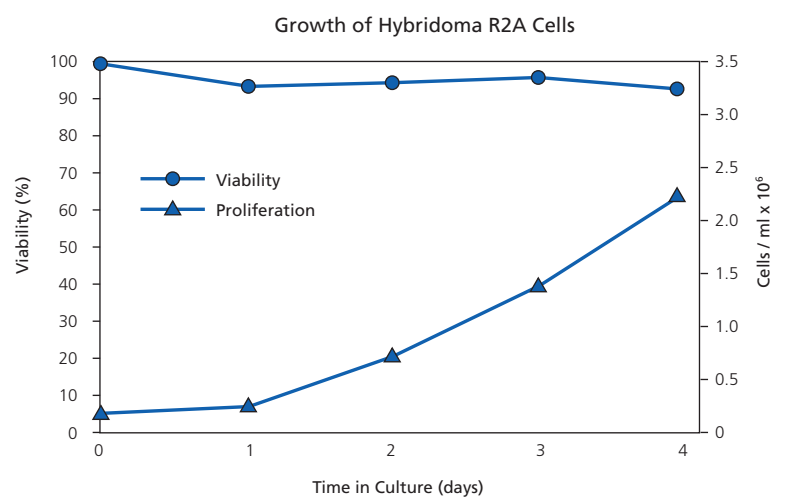
- ▶ Suitable for use in fermenters and roller bottles
- ▶ Increases the proliferation of hybridoma and NS0 cells
- ▶ Long-term cultivation

### Features

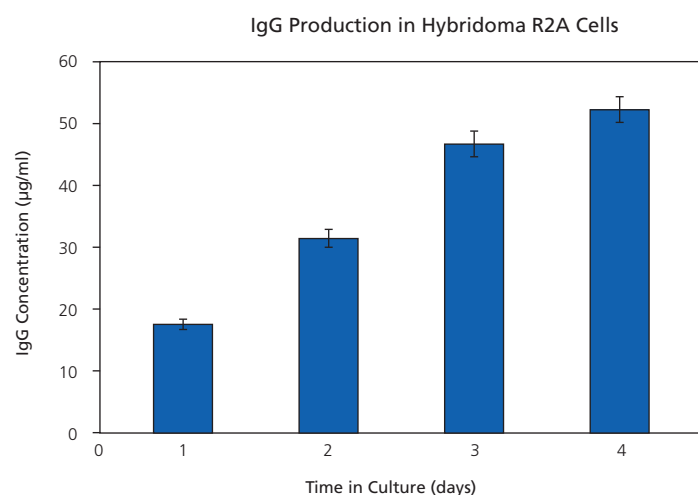
- ▶ Animal component free (ACF)
- ▶ Greatly increased antibody production
- ▶ Low protein content (5 µg/ml)
- ▶ Facilitated antibody purification
- ▶ Ready-to-use
- ▶ Work and time saving

### Order Information

|               |         |        |
|---------------|---------|--------|
| Hybridoma Pro | U15-077 | 500 ml |
|---------------|---------|--------|



The growth kinetics of Hybridoma R2A cells cultured in Hybridoma Pro medium show excellent proliferation and viability.



Hybridoma R2A cells cultured in Hybridoma Pro medium produced high antibody titres of 53 µg/ml on day 4.

# JuLI™

## The Fast, Convenient and Simple Answer to Live Cell Imaging

Live cell imaging is the method of choice for studying the cellular function of cells. Currently, to achieve this goal, complex and expensive set-ups are required, which unfortunately can increase cell stress.

JuLI™ has been designed for easy performance of a wide variety of live cell imaging experiments, without causing undue stress to cells. Fluorescent live cell images can be directly captured inside a tissue culture hood or a cell incubator using various cell culture dishes. Importantly, this prevents contamination and avoids changes to the cellular environment that can detrimentally affect cells, providing highly reliable and accurate results every time.

Store your sequential time-lapse fluorescence and/or bright-field images and convert them into movie files. With the automated capturing feature, JuLI™ performs your overnight imaging while you sleep.

### Applications

- ▶ Live cell imaging (time lapse)
- ▶ Cell migration assay
- ▶ Cell-based assay optimization
- ▶ Proliferation assay
- ▶ Cell culture quality control

### Features

- ▶ Simple and convenient operation
- ▶ No darkroom necessary
- ▶ Fluorescent and bright-field images
- ▶ Can be used directly in the incubator or tissue culture hood



### Order Information

|       |            |
|-------|------------|
| JuLI™ | W0001,0011 |
|-------|------------|

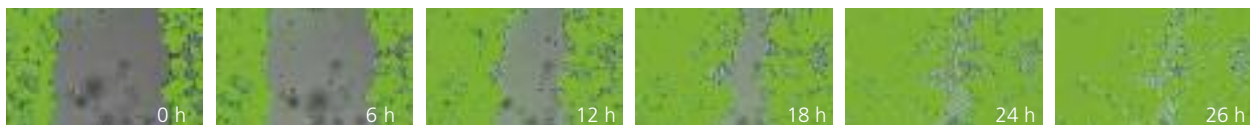




### Scratch Assay

**Procedure:** Cells were transfected with a GFP-labeled protein known to participate in cell-cell interaction processes at a transfection rate of approx. 80%. Confluent cultures of these cells were then disturbed in a scratch assay to monitor proliferation of the cells into the gap with JuLI™.

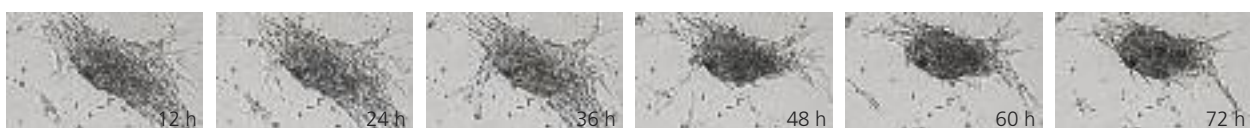
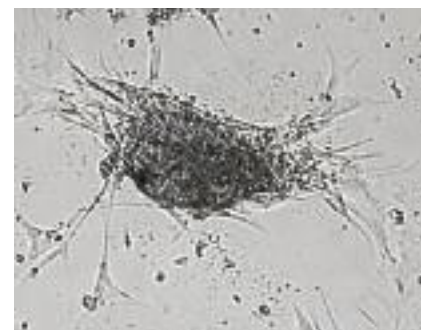
**Result:** Cells expressing the GFP-labelled protein are less likely to proliferate into the gap proposing a regulatory role in cell-contact mediated proliferation.



### Directed Migration Assay

**Procedure:** A culture dish was populated with small beads containing a putative chemoattractant. Then cells were plated on this dish at a confluency of approx. 20%. Subsequently cells were monitored with JuLI™ over 4 days.

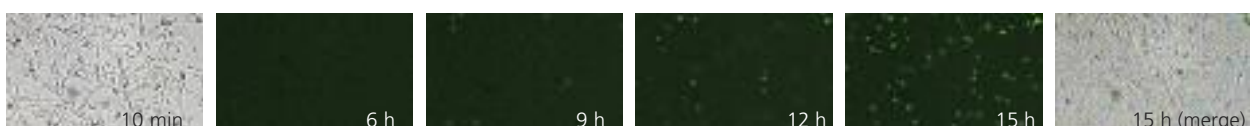
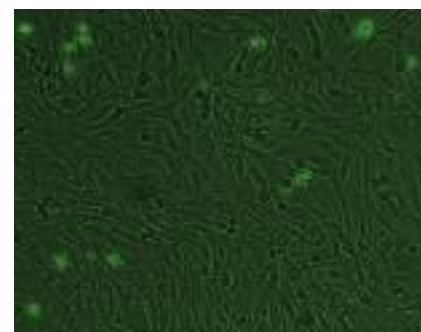
**Result:** Cells migrate towards the beads. Direct contact with the bead enforces the attraction effect.



### Expression Monitoring

**Procedure:** Cells were transfected with a GFP-labeled protein and monitored with JuLI™ over 16 hours to determine the starting point of expression.

**Result:** Expression of the protein started approx. 8 hours after transfection. First cells expressing the GFP-labeled proteins displayed strong fluorescence signal 12 hours after transfection.





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