BioResearch

Lonza

Metabolic Research Tools for Biologically Relevant Results

Cell Culture, Transfection and Analysis Tools for Metabolic Research

Avoid Research Roadblocks and Take a Direct Route to Results

"I'm uncertain of the results that I get from genetically modified cell lines."

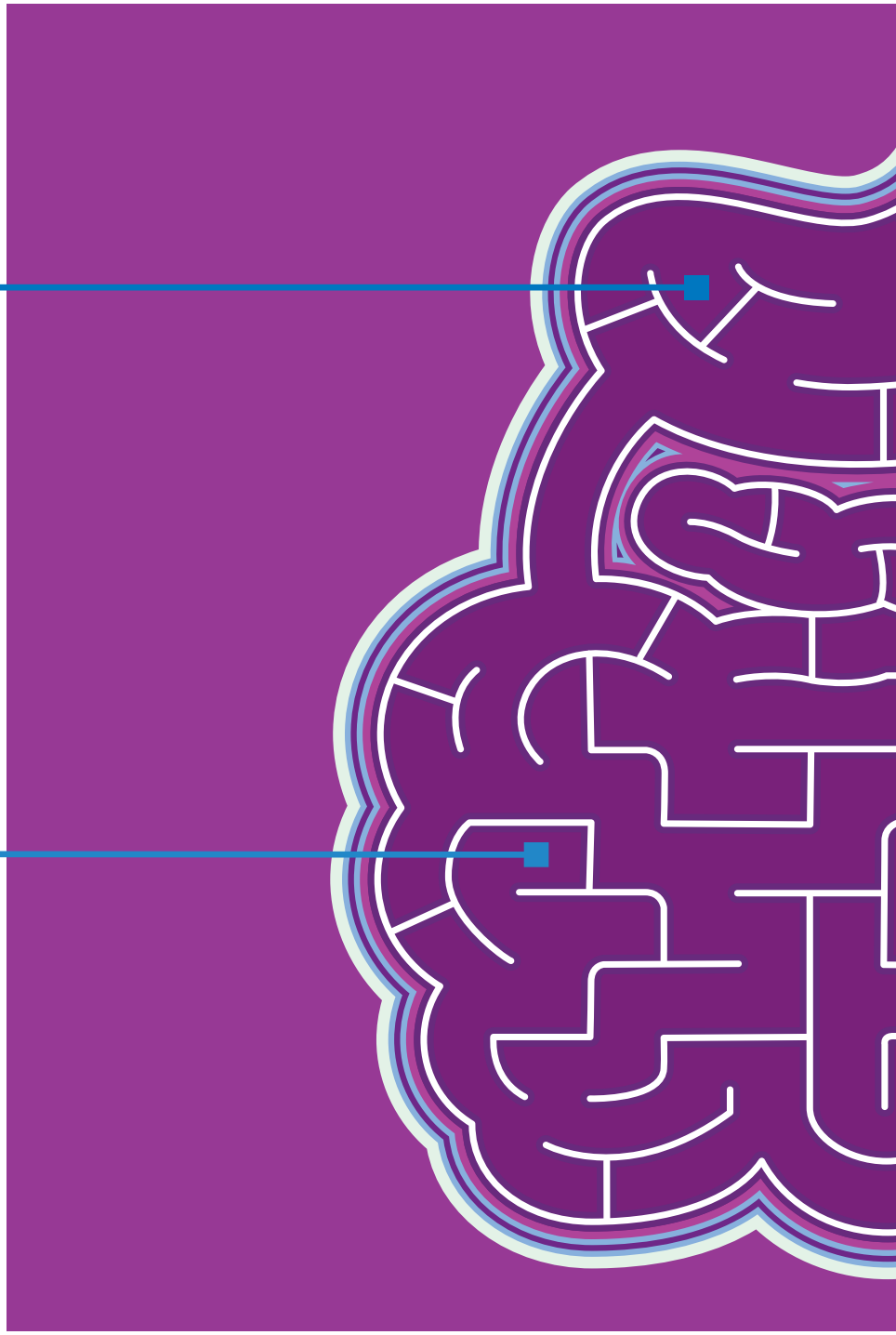
SOLUTION: Simulate *in vivo* research in culture by choosing from our comprehensive selection of primary cells. **Page 5**

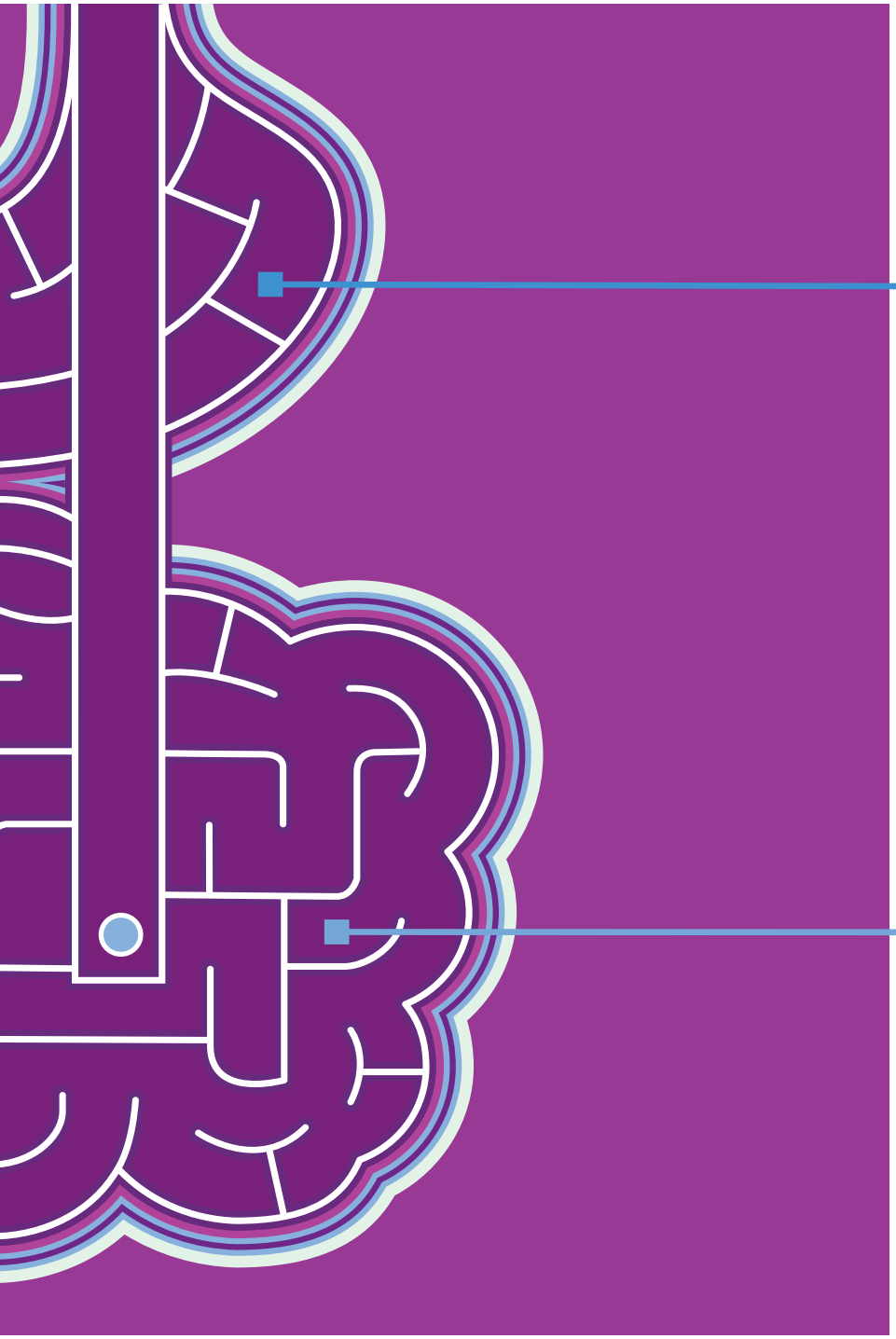
Primary Cells and Media

" I can't transfect my primary cells or cell lines of interest efficiently."

SOLUTION: Our Nucleofector™ Technology delivers up to 90% gene transfection efficiency, at high viability and maintenance of functionality. **Page 8**

Transfection





"How can I optimize my diabetic drug leads?"

SOLUTION: Lonza can ease your drug discovery research with our advanced cell culture tools such as 3D culture systems and Cells on Demand™ Cell Culture Services. **Page 10**

Drug Discovery

"I can't find reliable assay tools for protein expression, cell proliferation, or adipogenic cell studies."

SOLUTION: Now you can choose from a range of molecular biology reagents and assays developed specifically for diabetes and metabolic disorders. **Page 19**

Cell Analysis

Your Diabetes and Metabolic Toolkit

Streamline your workflow by choosing from convenient, innovative research tools that have been designed and tested to work together.

Lonza gives you what you need for biologically relevant results, from high-quality primary cells, through efficient transfection technology, to a wide range of analysis tools.

Primary Cells and Media

Normal and diabetic cryopreserved primary human cells:

Diabetic and normal human donor cell types:
- Aortic smooth muscle and endothelial cells
- Coronary artery smooth muscle and endothelial cells
- Pulmonary artery smooth muscle and endothelial cells
- Cardiac and dermal microvascular endothelial cells
- Renal proximal tubule epithelial cells
- Adult epidermal keratinocytes
- Skeletal muscle myoblasts
- Preadipocytes, subcutaneous and visceral
- Adult adipose derived stem cells

Other normal human donor cell types:
- Pancreatic Islets via Cells on Demand™ Service
- Renal cortical epithelial cells
- Renal epithelial cells
- Mesangial cells

Optimized Clonetics™ Primary Cell Growth Media:
- EGM™ 2 Endothelial Growth Media
- SmGM™ 2 Smooth Muscle Growth Media
- REGM™ Renal Growth Media
- MsGM™ Mesangial Growth Media
- KGM™ Gold Keratinocyte Growth Media
- PGM™ 2 Preadipocyte Growth Media
- ADSC Growth and Differentiation Media
- HCM™ Hepatocyte Culture Media

Transfection

Nucleofector™ Technology with optimized protocols for primary cells including:
- Endothelial cells, such as aortic and coronary artery
- Epithelial cells, such as human kidney epithelial cells
- Smooth muscle cells, such as aortic smooth muscle
- Rat, mouse and human hepatocytes
- Human preadipocytes

Also optimized for many cell lines, including:
- Hep G2
- Capan-1
- HCT 116
- Ins-1
- Min6
- MDCK

Drug Discovery

RAFT 3D Cell Culture System
- Cancer Research
- Dermal Research
- Corneal Models
- Blood Brain Barrier Models

Conditionally immortalized human cell lines:
- Preadipocytes
- Skeletal Muscle Cells
- Dermal Fibroblasts
- Coronary Artery Smooth Muscle Cells (CASMC)
- Blood (BEC) and Lymphatic (LEC) Microvascular Endothelial Cells
- Adult Dermal Keratinocytes
- Brain Microvascular Endothelial Cells

Biowhittaker™ Liquid and Powder Media for therapeutic and research needs

Cells on Demand™ Services:
- Custom transfection services
- Custom cell isolation services

Cell Analysis

Cell proliferation and cytotoxicity bioassay kits:
- ViaLight™ Kit for measuring cell proliferation and cytotoxicity
- ToxiLight™ Kit for non-destructive measurement of cytotoxicity

Cell function assays:
- Bone mineralization and resorption assays
- Adipogenesis and lipolysis measurement assays

Mycoplasma detection:
- MycoAlert™ Assay for accurate, reliable and universal mycoplasma detection

DNA, RNA, and protein expression analysis:
- PAGEr™ EX Gels and ProSieve™ EX Reagents for protein analysis and Western blotting
- FlashGel™ System for PCR analysis and RNA integrity analysis

Living Cell Monitoring
- CytoSMART™ System: small, easy and affordable live cell imaging system for documentation or monitoring of cell cultures

Primary Cells and Media

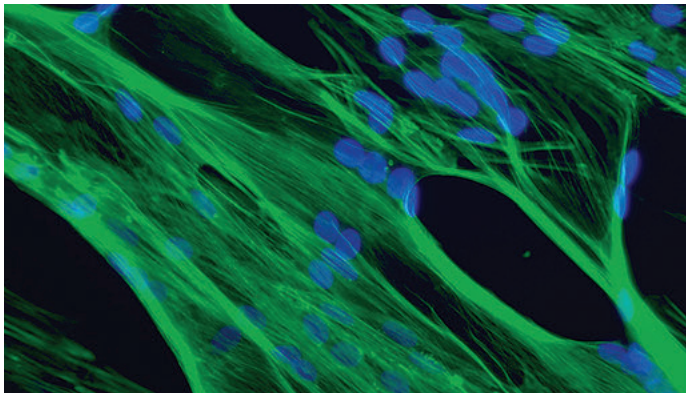
Choose from a wide range of high-quality primary, non-transformed, non-immortalized cells from Lonza. They have all been tested and matched with media to save you the time and effort of optimization.

More than 20% of cell culture studies are based on misidentified or cross-contaminated cell lines. Lonza's products have been developed to give you complete confidence in your results.

Our cells have been sourced from a variety of donors, including those diagnosed with diabetes type I and diabetes type II.

"I'm uncertain of the results that I get from genetically modified cell lines."

SOLUTION: Simulate *in vivo* research in culture by choosing from our comprehensive selection of primary cells.



Human skeletal muscle myoblasts, differentiated and immunostained with desmin, counterstained with DAPI

Clonetics™ Diabetic Cells

Enhance the relevance of your results by working with human primary cells that have been isolated from diabetes type I and II donors:

- Compare diseased cells to normal primary cells for a better understanding of effects
- To learn more about cell donors, contact our scientific support team
- All cells test negative for bacterial, fungal, and mycoplasma contamination
- Human cells test negative for HIV-1, Hepatitis-B, and Hepatitis-C

Diabetic human cells include:

- Aortic smooth muscle cells
- Coronary artery smooth muscle cells
- Pulmonary artery smooth muscle cells
- Aortic endothelial cells
- Coronary artery endothelial cells
- Pulmonary artery endothelial cells
- Cardiac microvascular endothelial cells
- Dermal microvascular endothelial cells
- Renal proximal tubule epithelial cells
- Adult epidermal keratinocytes
- Skeletal muscle myoblasts
- Preadipocytes, subcutaneous and visceral
- Adult adipose derived stem cells

For best results, match these cells with our optimized Clonetics™ Media BulletKits™.

More primary cells and media on the next page.

Normal Human Cells

Pancreatic Cells
- Fresh human pancreatic islets

Smooth Muscle Cells
- Aortic smooth muscle cells
- Coronary artery smooth muscle cells
- Pulmonary artery smooth muscle cells

Endothelial Cells from Large Vessels
- Aortic endothelial cells
- Coronary artery endothelial cells
- Pulmonary artery endothelial cells
- Iliac artery endothelial cells
- Umbilical vein endothelial cells, single and pooled
- Umbilical Vein endothelial cells, expanded

Endothelial Cells from Small Vessels
- Cardiac microvascular endothelial cells
- Dermal microvascular endothelial cells, adult and neonatal
- Dermal lymphatic microvascular endothelial cells, adult and neonatal
- Lung blood microvascular endothelial cells
- Lung microvascular endothelial cells
- Lung lymphatic microvascular endothelial cells

Renal Cells
- Renal proximal tubule epithelial cells
- Renal cortical epithelial cells
- Renal epithelial cells
- Mesangial cells

Intestinal Cells
- Myofibroblasts
- Epithelial cells

Dermal Cells
- Adult epidermal keratinocytes
- Neonatal epidermal keratinocytes, single and pooled donors

Skeletal Muscle Cells
- Skeletal muscle myoblasts
- Skeletal muscle cells

Adipose Cells
- Preadipocytes, subcutaneous and visceral
- Adult adipose derived stem cells

Clonetics™ Media BulletKits™

- EGM™ 2 Endothelial Growth Media
- SmGM™ 2 Smooth Muscle Growth Media
- REGM™ Renal Growth Media
- MsGM™ Mesangial Growth Media
- KGM™ Gold Keratinocyte Growth Media
- PGM™ 2 Preadipocyte Growth Media
- ADSC Growth and Differentiation Media
- HCM™ Hepatocyte Culture Media

You can find recommended media with the Ordering Information on page 24.

Primary Cells for Validation of Cell Line Results

Find the metabolism-related cell line you're working with below and the related primary cell type to validate those results.

Cell Line	Recommended Primary Cell from Lonza
3T3-L1 mouse fibroblast	Human preadipocytes, visceral or subcutaneous
L6 and C2C12 Rat muscle myoblast cell line and mouse muscle myoblast cell line	Primary human skeletal muscle myoblasts
Caco-2 Human intestinal epithelial cell line	Primary human intestinal myofibroblasts and epithelial cells
ECV-304 (This line was originally thought to be from a spontaneously-transformed line derived from a Japanese human umbilical vein endothelial cells however it was found to be human bladder cancer derived epithelial cell line T24/83)	Primary human umbilical vein endothelial cells (HUVEC)
INS-1, RIN5F, MIN6	Rat and mouse cell lines as substitutes for beta cells
Others	Call Lonza scientific support for additional primary cell types that match your need for biological relevance

Primary Cell Case Study: Adipogenesis and Lipolysis in Differentiated Human Subcutaneous Adipocytes

Measuring adipogenesis and lipolysis in human subcutaneous adipocytes using new sensitive, biochemical assays
Marjorie Smithhisler and Huan Tran from Lonza Walkersville, Inc., USA

Summary

The physiological functions associated with fat metabolism are still being elucidated. However, it is known that the accumulation and break-down of triglycerides cause widespread systemic effects. Two most commonly studied sources of adipocytes are from visceral and subcutaneous fat tissue. Subcutaneous fat is often found attached to skin in the lower abdomen area. Visceral Preadipocytes are isolated from adipose tissue associated with internal organs, such as the bladder or kidney. Relative to subcutaneous fat, visceral fat deposits are mobilized at a higher rate to produce fatty acids that contribute to insulin resistance, Diabetes Type 2, and other related cardiovascular disorders. Measuring the increase and break-down of intracellular triglycerides into free fatty acids and glycerol within adipocytes is important to discovering new cures for a myriad of metabolic disorders.

Methods and Materials

Subcutaneous and visceral preadipocytes (Lonza) were grown and differentiated per cell system instructions in PGM™ 2 Preadipocyte Growth Medium at 37°C, 5% CO_2. Differentiation media was added and cells were monitored for lipid accumulation over 10 days. Cells were then stained with AdipoRed™ Adipogenesis Assay Reagent. Cells were induced to undergo lipolysis via the addition of 1 µM isoproterenol and induction was tested for insulin inhibition with 10 µg/mL insulin addition. Cell supernatants were diluted and tested with the AdipoLyze™ Lipolysis Detection Assay for glycerol accumulation. For more detailed information about the cells, assays, or lipolysis induction contact Lonza scientific support.

Results

Subcutaneous and visceral preadipocytes both showed marked lipid accumulation after 10 days via AdipoRed™ Staining (Figure 1, quantitative plate reader results not shown). Both cell types showed increased levels of lipolysis in the differentiated cultures versus undifferentiated cultures as determined by AdipoLyze™ assay, which measures extracellular release of glycerol, a by-product of triglyceride breakdown. Both cultures showed lipolysis inhibition by the addition of insulin (Figure 2, subcutaneous data only shown here).

Conclusions

In summary, primary visceral and subcutaneous preadipocytes showed both the accumulation and breakdown of intracellular lipid, and these two processes were adequately measured via AdipoRed™ and AdipoLyze™ cell-based assays. The cells, media and assays can be used congruently to study certain metabolic changes *in vitro* research studies.

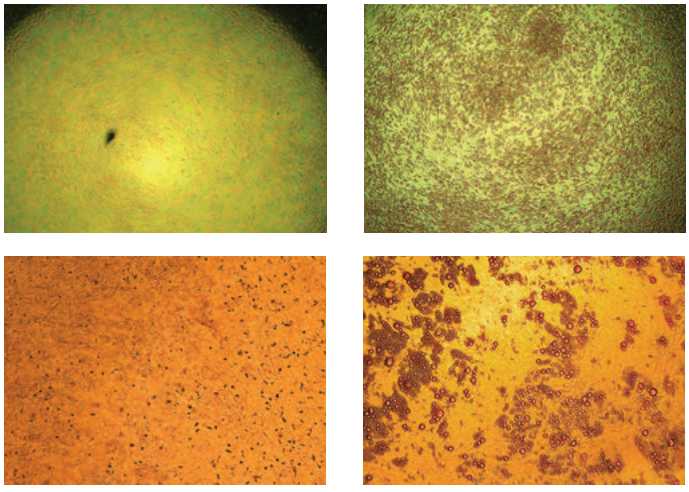


Figure 1. Subcutaneous (top) and visceral (bottom) preadipocytes (undifferentiated, left column, differentiated, right column) stained with AdipoRed™ assay reagent.

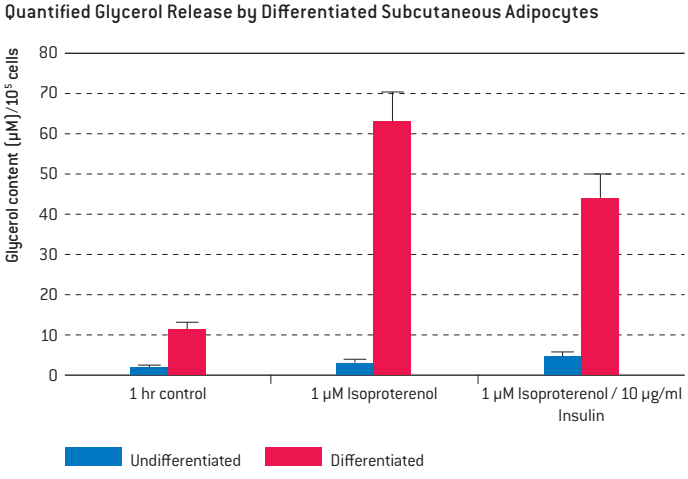


Figure 2. AdipoLyze™ Lipolysis Detection Assay results for glycerol accumulation in subcutaneous preadipocytes.

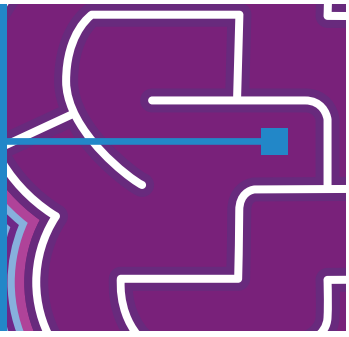
Transfection

You can deliver almost any substrate – such as plasmid DNA, siRNA, shRNA, or peptides – to primary cells and hard-to-transfect cell lines with our non-viral Nucleofector™ Technology.

This improved technique will play an increasingly important role in delivering genes to primary smooth muscle, epithelial, and endothelial cells.

" I can't transfect my primary cells or cell lines of interest efficiently."

SOLUTION: Our Nucleofector™ Technology delivers up to 90% gene transfection efficiency, at high viability and maintenance of functionality.



Nucleofector™ Technology

Transfect primary cells and cell lines related to metabolic diseases with up to 90% efficiency while maintaining excellent cell viability. Nucleofection™ allows you to:

- Use validated transfection protocols for 13 primary cells related to metabolic diseases
- Select optimized transfection conditions for more than 30 cell lines related to metabolic diseases
- Easily establish Nucleofection™ conditions for other mammalian cells using our cell primary cell or cell line optimization protocols
- Preserve cellular function and reduce toxicity
- Use the same transfection conditions for different cell numbers and volumes
- Select from a range of platforms to suit your cell number and throughput needs

Choose the Nucleofector™ Platform that Suits Your Research Needs

	Advanced Platform	96-Well Add-On	High-Throughput Platform	Basic Device
Device	4D-Nucleofector™ System	96-well Shuttle™ System	384-well Nucleofector™ System	Nucleofector™ 2b Device
				
Unit				
Throughput (samples per run)	Low to medium (1 – 16)	Low to high (1 – 96)	High (384)	Low (1)
Reaction volume	20 µL + 100 µL	20 µL	20 µL	100 µL
Electrode material	Conductive polymer	Conductive polymer	Conductive polymer	Aluminum
Low cell numbers (20 µL)	5×10^4 to 1×10^5	5×10^4 to 1×10^5	5×10^4 to 1×10^5	—
High cell numbers (100 µL)	2×10^5 to 2×10^6	—	—	2×10^5 to 2×10^6
DNA vector amount/sample	0.2 – 1 µg (20 µL) 1 – 5 µg (100 µL)	0.2 – 1 µg	0.2 – 1 µg	1 – 5 µg
siRNA amount/sample (concentration 2 nM – 2 µM)	0.04 – 40 pmol (20 µL) 0.2 – 200 pmol (100 µL)	0.04 – 40 pmol	0.04 – 40 pmol	0.2 – 200 pmol
Compatibility with 96-well Shuttle™ System	■	—	—	—

www.lonza.com/celldatabase – To find transfection data for your cell type of interest.

Transfection Efficiency and Cell Viability with Nucleofector™ Technology

	Efficiency*	Viability*	Kit for 4D-Nucleofector™ and 96-Well Shuttle™ Systems (Name of Specific Protocol)	Kit for Nucleofector™ II/2b Device
Smooth Muscle Cells				
SMC, aortic (AoSMC), human	75-80%	55-95%	P1 Primary Cell Kit (AoSMC)	AoSMC Nucleofector™ Kit
SMC, coronary artery (CASMC), human	60-70%	80-90%	P1 Primary Cell Kit (smooth muscle cell, basic)	Basic Smooth Muscle Cell Nucleofector™ Kit
SMC, pulmonary artery (PASMC), human	75-85%	60-70%	P1 Primary Cell Kit (smooth muscle cell, basic)	Basic Smooth Muscle Cell Nucleofector™ Kit
Endothelial Cells, Large Vessels				
Aortic endothelial cells, human	55-75%	45-60%	P5 Primary Cell Kit (endothelial cell, basic)	Basic Endothelial Cell Nucleofector™ Kit
Coronary artery endothelial cells, human	50-60%	75-100%	P5 Primary Cell Kit (endothelial cell, basic)	HCAEC Nucleofector™ Kit
Endothelial, umbilical vein, human (HUVEC)	80-90%	55-75%	P5 Primary Cell Kit (HUVEC)	HUVEC Nucleofector™ Kit
Endothelial Cells, Small Vessels				
Dermal microvascular endothelial cells, human	64%	99%	P5 Primary Cell Kit (endothelial cell, basic)	Basic Endothelial Cell Nucleofector™ Kit
Lung microvascular endothelial cells, human	79%	48%	P5 Primary Cell Kit (endothelial cell, basic)	HMVEC-L Nucleofector™ Kit
Renal Cells				
Mesangial cells, human	83%	81%	Primary Cell Optimization Kit	Basic Smooth Muscle Cell Nucleofector™ Kit
Intestinal Cells				
Myofibroblasts, human	57%	no data	P2 Primary Cell Kit (fibroblast, basic) P3 Primary Cell Kit (fibroblast, basic)	Basic Fibroblast Nucleofector™ Kit
Dermal Cells				
Adult epidermal keratinocytes, human	60%	62%	P3 Primary Cell Kit (adult keratinocytes)	Human Keratinocyte Nucleofector™ Kit
Neonatal epidermal keratinocytes, human	63%	67%	P3 Primary Cell Kit (adult keratinocytes)	Human Keratinocyte Nucleofector™ Kit
Adipose Cells				
Preadipocytes, subcutaneous and visceral, human	33-92%	42-81%	P1 Primary Cell Kit	Basic Fibroblast Nucleofector™ Kit
Adult adipose derived stem cells, human	73-84%	58-85%	P1 Primary Cell Kit	Human MSC Nucleofector™ Kit
Hepatocytes				
Hepatocytes human	54%	59-69%	P3 Primary Cell Kit (hepatocytes, human)	Contact scientific support for guidance
Hepatocytes, mouse	54%	80%	Primary Cell Optimization Kit	Mouse/Rat Hepatocyte Nucleofector™ Kit
Hepatocytes, rat	52%	78%	Primary Cell Optimization Kit	Mouse/Rat Hepatocyte Nucleofector™ Kit
Kidney/Renal Related Cell Lines**				
MDCK	83-98%	51-95%	SE Cell Line Kit	Cell Line L
BHK-21	85%	78%	SE Cell Line Kit	Cell Line L
Pancreas Related Cell Lines**				
Min6	60-75%	40-80%	Cell Line Optimization Kit	Cell Line T
Ins-1	70-90%	75-85%	Cell Line Optimization Kit	Cell Line T
Capan-1	29-67%	40-78%	SE Cell Line Kit	Cell Line V
Gastro Intestinal Related Cell Lines**				
Colo201	57%	78-96%	Cell Line Optimization Kit	Cell Line R
HCT 116	78-94%	61-76%	SE Cell Line Kit	Cell Line V
Liver Related Cell Lines				
Hep-G2	72-95%	92%	SF Cell Line Kit	Cell Line V

Primary cells marked **blue** have Lonza-validated optimized protocols.

*Approximate ranges extrapolated from larger result collections, including Lonza and customer data

**This is only a selection of cell lines. www.lonza.com/celldatabase – For further cell lines and protocol guidance.

Drug Discovery

Our dedicated drug discovery team gives you access to Lonza's expertise in many areas of cell biology. We trust that we have identified the products and services of value to drug discovery customers and we welcome the opportunity to discuss and develop them further with you.

RAFT™ 3D Cell Culture System

3D cell culture differs significantly from the traditional 2D culture that most researchers have been using since the past decades. There is a trending shift both in academia and industry to personalized research solutions and more *in vivo* like models to understand cell behavior. This is fueling the growing market need for better solutions in 3D cell culture such as RAFT™ 3D Cell Culture System.

An important differentiation of the RAFT™ System to other 3D platforms or to 2D culture methods is its ability to engineer tissue-like 3D cultures. This is especially needed for certain applications such as development of *in vitro* liver fibrosis models or corneal models. With RAFT™ System, cells can be cultured within a **high-density** collagen scaffold, or on top, or both. The addition of permeable membrane cell culture inserts provides other extensions to the system allowing the generation of barrier models including air-lift models.

RAFT™ Cultures can be created in less than an hour using simple protocols and standard labware! RAFT™ Kits are available in a choice of 24-well, insert-well and 96-well formats. RAFT™ System has already been used to successfully generate 3D cultures in a number of research areas including oncology, toxicology, barrier modeling, dermal research and pulmonary research.

Contact your local sales representative to learn how RAFT™ System can support your experiments.

Figure 1. RAFT™ 3D Culture System consists of RAFT™ Reagent Kit and Absorbers for 96-well, 24-well and trans-well inserts.

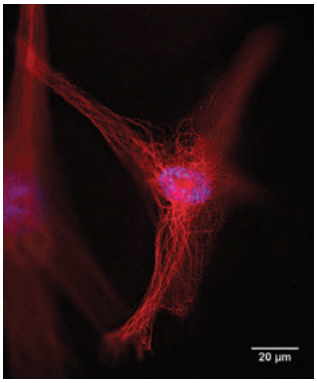


Figure 2. Dermal fibroblasts fixed and stained after 11 days in RAFT™ System.

Figure 3. Sample acellular RAFT™ Cultures in a vial showing high-density collagen scaffolds.

Fluorescence Microscopy-based Characterization of Cells Grown in RAFT™ 3D Culture

By Cecile Villemant[1], Grant Cameron[1], Lubna Hussain[2], Jenny Schroeder[3]
[1]TAP-Biosystems, Royston, UK; [2]Lonza Walkersville, Inc., Walkersville, MD, USA; [3]Lonza Cologne GmbH, Koeln, Germany

Introduction

In vitro assays typically use cells grown on two-dimensional (2D) hard plastic or glass substrates, which are not representative of the true *in vivo* cell environment.[1] In tissue, cells interact with neighboring cells and with the extracellular matrix (ECM). In a simplified *in vitro* 2D environment, most of the tissue-specific architecture, cell-cell communication and cues are lost. Therefore, the need exists for advanced culture methods that better mimic cellular function within living tissue.

Three-dimensional (3D) cell culture methods, in comparison, provide a matrix that encourages cells to organize into structures more indicative of the *in vivo* environment, thereby developing normal cell-cell and cell-ECM interactions in an *in vitro* environment.

The RAFT™ 3D Culture System (Figure 1) uses a collagen matrix at physiologically relevant concentrations. Cells and neutralized collagen are mixed and dispensed into wells of standard cell culture plates, and subsequently incubated at 37°C to allow the formation of a cell-seeded hydrogel. Specialized RAFT™ Absorbers are placed on top of the hydrogels. The RAFT™ Absorbers gently remove the medium, thus concentrating the cell/collagen hydrogel to a layer approximately 120 µm thick, mimicking physiological conditions. The cultures are then ready for use. Optionally, additional collagen layers or epithelial or endothelial cell overlays may be added to study co-cultures or more complex cultures.

Assessing the viability of cells grown in a RAFT™ 3D Cell Culture, or performing immunofluorescence staining, may not seem as trivial as assessing the viability of a 2D cell culture. However, here we show that by adding some basic controls, the LIVE/DEAD® Assay is a straightforward and reliable method to assess the viability of cells inside a RAFT™ 3D Cell Culture. In addition, we show that immunofluorescence microscopy can be performed easily and routinely on RAFT™ 3D Cell Cultures.

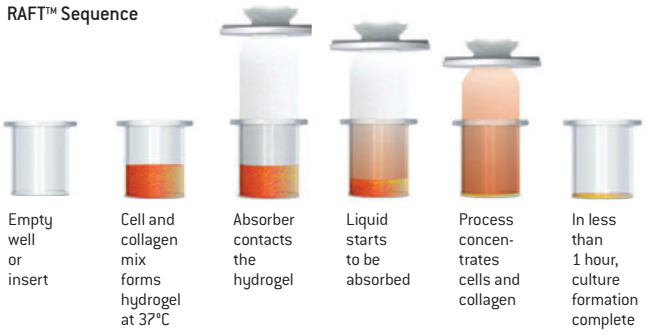


Figure 1. Creation of 3D cell/collagen hydrogel using the RAFT™ System in standard cell culture plates.

General Materials

- RAFT™ Absorbers and Reagent Kits, visit **www.lonza.com/raft** for a list of RAFT™ Products and RAFT™ Protocols
- For a list of recommended cell culture plates (either 96-well black wall or 24-well plate, not supplied with the kit), contact Lonza's Scientific Support Team
- Widefield fluorescence microscope with appropriate filters

Methods

Cell Culture
Early passage primary human neonatal dermal fibroblasts (HDFs) were cultured in standard growth medium that supports dermal fibroblasts.

Generation of RAFT™ Cultures
RAFT™ Cultures were made according to the protocol supplied with the RAFT™ Kits in black-walled, µClear® 96-well cell culture plates (Greiner, 655090). For the LIVE/DEAD® Assay, 3,000 HDFs per well were seeded and incubated at 37°C with 5% CO_2, either overnight (1 day) or for 7 days. In addition, two acellular cultures were made for use as a background control. For immunocytochemistry, either 5,000 or 50,000 HDFs per well were seeded and then cultured for either 11 days or 3 days, respectively, prior to being fixed and stained.

Methods continued

LIVE/DEAD® Assay

The LIVE/DEAD® Assay was carried out, using at least one dead control and two background controls (acellular cultures). The LIVE/DEAD® Viability/Cytotoxicity Kit (Life Technologies) was used according to the "fluorescence microscopy protocol" provided up to point 3.5. However, with the following adjustments:

1. To get a thorough wash of the whole 3D cell culture, the medium on the culture is aspirated and replaced with 100 µL PBS and the plate left for 5–10 minutes on a rocker (while preparing the combined LIVE/DEAD® Assay Reagents).
2. To prepare the dead cell sample, we incubate the cells with 1% w/v saponin for at least 30 minutes. If you have added 100 µL of medium onto your RAFT™ Cultures, just add 25 µL of 5% w/v saponin 30 minutes before performing the assay.
3. Due to the presence of the collagen matrix, it is advised to include acellular RAFT™ 3D Cultures to act as a control for background noise.

Instead of point 4.1, onward, in the supplied LIVE/DEAD® Viability/Cytotoxicity Kit Protocol, we used the following protocol:

Aspirate the PBS, added previously from the wells, and replace it with 100 µL of the combined LIVE/DEAD® Assay Reagents. We have found that in the case of HDFs, final concentrations of 0.4 µM for Calcein AM and 4 µM for Ethidium homodimer 1 (EthD-1) were optimal.

Between 10 minutes and 1.5 hours after adding the reagents, z-series at 5 µm intervals were captured using a fluorescence microscope fitted with a z-focus drive. The numbers of live cells (stained with Calcein AM) and dead cells (stained with EthD-1) were counted from three separate images over two wells for the "live" samples. The background noise for EthD-1 was counted in two acellular cultures ($bckgd_{EthD\text{-}1}$① and $bckgd_{EthD\text{-}1}$②). The background noise for Calcein AM was assessed in the acellular cultures and in the dead control; however, there was no visible background detected. Therefore, for each image, the percentage of viability was calculated as follows:

$$\%viability_{d=1 \text{ or } d=7} = \frac{\text{Number of live cells}}{\text{Number of live + dead cells} - \text{average } (bckgd_{EthD\text{-}1}\ ①\&②)}$$

Immunocytochemistry

On the day of assay, the following protocol was used:

1. Each RAFT™ Culture was washed three times over a 15-minute period with 100 µL PBS.
2. The PBS was replaced with 100 µL of 3.7% formaldehyde solution to fix the cells, and the plate incubated at room temperature for 30 minutes.
3. The formaldehyde solution was replaced with 100 µL of quenching solution (1 mM Tris-HCl and 20 mM Glycine in PBS) to quench the formaldehyde cross-linking, and the plate incubated at room temperature for 10 minutes.
4. The RAFT™ Cultures were washed as in point 1.
5. The PBS was replaced with 100 µL of 0.1% Triton™ X-100 solution to permeabilize the cells, and the plate was incubated at room temperature for 4 minutes.
6. The RAFT™ Cultures were washed as in point 1.
7. The primary rat anti-tubulin antibody (YOL1/34; Abcam) was diluted 1:100 in 1% w/v bovine serum albumin in PBS (which was the same dilution that was optimal for cells cultured in 2D) and 50 µL of this solution was added in each well.
8. The plate was then incubated overnight at 4ºC.
9. The RAFT™ Cultures were washed as in point 1.
10. The secondary antibody Cy3-AffiniPure Goat Anti-Rat IgG (H+L) (Stratech Scientific), phalloidin (the recommended 1/40 dilution was used) and DAPI were diluted in 1% w/v bovine serum albumin in PBS and 50 µL of this solution was added in each well.
11. The plate was then incubated at room temperature for 2.5 hours.
12. The RAFT™ Cultures were washed as in point 1.
13. The wells were imaged on a fluorescence widefield microscope with 100–200 ms exposure times for the anti-tubulin antibody and the phalloidin. Confocal imaging or the use of a high content imaging device would also be possible.

Results

LIVE/DEAD® Assay

In Figure 2, we show the typical images that can be obtained after culturing HDFs in RAFT™ 3D Cell Cultures for 1 and 7 days and staining the cultures with the combined LIVE/DEAD® Assay Reagents. We also show, in Figure 2, some examples of the Calcein AM and EthD-1 stain on an acellular construct and a dead control.

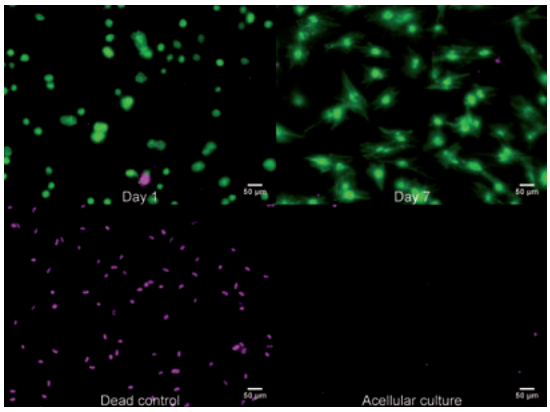


Figure 2. Examples of images that can be taken after staining RAFT™ Cultures with the combined LIVE/DEAD® Assay Reagents. HDF live cells are displaying Calcein AM staining (green) while dead cells display EthD-1 staining (magenta). Each image is a projection on the z-axis of a whole z-series.
Day 1 and Day 7: HDFs were cultured for 1 or 7 days, respectively, in a RAFT™ Plate before being treated with the combined LIVE/DEAD® Assay Reagents.
Dead control: HDFs cultured in RAFT™ Plate for 1 day were killed using 1% w/v saponin before being stained with the combined LIVE/DEAD® Assay Reagents.
Acellular culture: RAFT™ Acellular Culture stained with the combined LIVE/DEAD® Assay Reagents.

Figure 3 shows that the mean percentage viability of HDFs is 92% at Day 1 and 94% at Day 7 using the methods described above. For comparison, we have added the viability observed with the same cells cultured at the same time in a 2D planar environment, which is at 95% and 98% at Day 1 and Day 7, respectively.

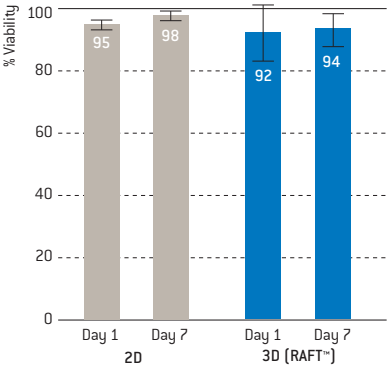


Figure 3. Comparison of the viability of HDFs after 1 and 7 days in a 2D or in a RAFT™ 3D Cell Culture. The percentage viability was determined as explained in the methods above and the average for two separate experiments is shown in this graph. The standard deviation is shown for each sample.

Immunocytochemistry

After 3 days in culture, HDFs have elongated within the collagen matrix and display a typical actin and microtubule cytoskeleton as can be seen in Figures 4 and 5. The presence of the collagen matrix has little impact on the background fluorescence of the culture, in particular, when the antibodies were diluted in a BSA-containing blocking buffer.

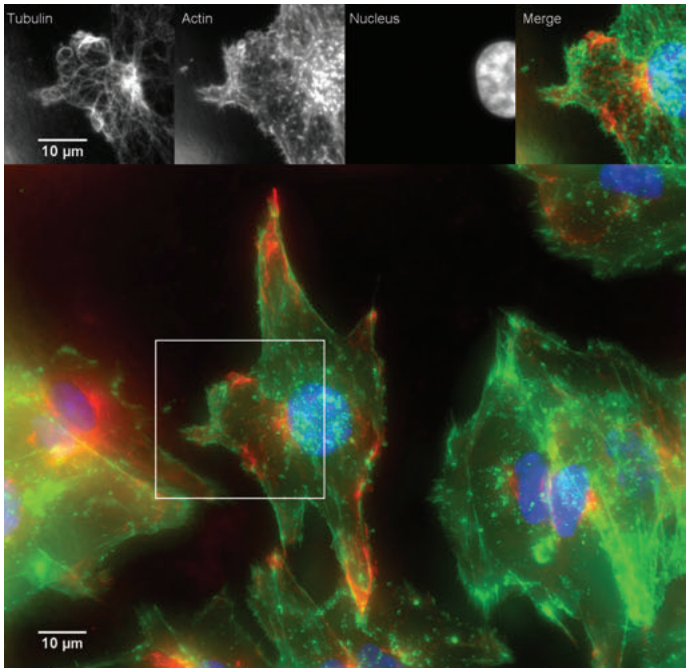


Figure 4. HDF cells fixed and stained for tubulin and actin after being cultured in the RAFT™ System for 3 days. A series of z-planes, taken at 0.5 µm intervals, was imaged on a widefield microscope after staining the RAFT™ Culture for tubulin (red), actin (green) and nucleus (blue). The z-stack from each channel was projected onto one plane using the maximum z-projection function of ImageJ software and the merge of all channels is shown in the large bottom panel. Across the top panel, one frame of the z-stack is shown with each individual channel represented separately in grayscale. This represents the area boxed in the large lower panel, to better show the detail of each staining.

Results continued

As shown in Figure 5, using a simple type of image deconvolution, such as ImageJ 3D parallel spectral deconvolution (http://rsb.info.nih.gov/ij/plugins/), can help improve the sharpness of the signal and remove background noise from the cells surrounding the cell of interest (such as the blurry, out of focus cell observed above the fibroblast imaged in Figure 4). However, with our anti-tubulin antibody, images taken with our widefield microscope, and not subjected to deconvolution, were defined enough to observe microtubules within the cells in the RAFT™ 3D Culture (Figure 4). Actin fibers can also be seen clearly in cells embedded in the RAFT™ Collagen Matrix (Figure 4).

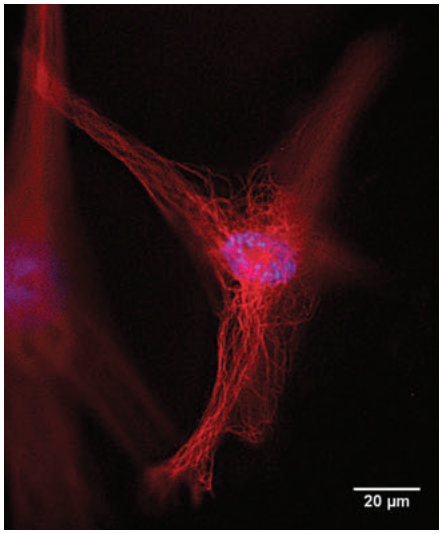


Figure 5. HDF cell fixed and stained for tubulin after being cultured in the RAFT™ System for 11 days. One z-plane location was imaged here on a widefield microscope, after staining the RAFT™ Culture for tubulin (red) and the nucleus (blue). The image from each channel was deconvolved using the 3D spectral deconvolution software from ImageJ (generalized Tikhonov) and the merge of the two channels is shown here.

Conclusion

The LIVE/DEAD® Viability/Cytotoxicity Kit is an easy and rapid assay that can be used to assess cell viability in a RAFT™ 3D Cell Culture, provided that controls are included in the test to be able to take into account the possible background noise. In this experiment, we show that HDFs display a mean viability between 92% and 94% when cultured in RAFT™ 3D Culture from Day 1 and for at least 7 days, which is comparable to the viability observed for cells cultured in a 2D environment.

Cells cultured in the RAFT™ 3D Cell Culture Collagen Matrix can be fixed and stained using standard immunofluorescence protocols. The presence of the collagen matrix has little impact on the background fluorescence of the culture, if an appropriate blocking solution is used. The resulting images were defined enough to observe microtubules and actin fibers within the cells in the RAFT™ 3D Culture. Image quality can be further enhanced by using simple types of image deconvolution software.

In conclusion, with its easy-to-follow protocols, the RAFT™ System allows researchers to set up biologically relevant 3D cell cultures quickly and reproducibly. Many 2D cell analysis methods can be easily applied to RAFT™ Cultures, often requiring no, or only minor modifications of existing protocols. This empowers researchers to generate more biologically meaningful data from their cell culture studies in multiple areas of basic research and drug discovery.

References

1. Pampaloni, F.; Reynaud, E.G.; Stelzer, E.H.K. The third dimension bridges the gap between cell culture and live tissue. *Nat Rev Mol Cell Biol.* 2007, 8, 839–845.

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- Human Dermal Fibroblasts
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- Human Adult Dermal Keratinocytes

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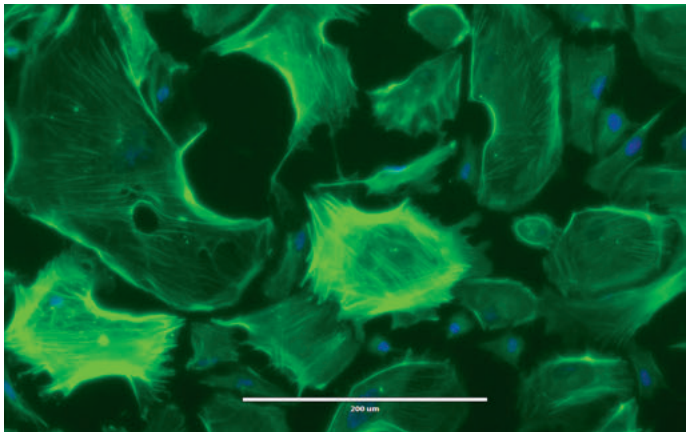

Figure 1. Podocytes at P4, 10k aSMA overlay (20x)

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- Stress-free optimization of Nucleofection™ Protocols for your cell type of interest

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- Avoid the aggravation of tissue acquisition, failed isolations, and low yields with our primary cell isolation service
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Diabetes-related Differential Gene Expression in Primary Human Adipose-derived Stem Cells and Aortic Endothelial Cells

By Rochelle Myers[1], Lubna Hussain[1] and Ludger Altrogge[2]
[1]Lonza Walkersville, Inc., Walkersville, MD, USA; [2]Lonza Cologne GmbH, Koeln, Germany

Introduction

With the prevalence of diabetes growing worldwide, the availability of primary human cells from diabetic donors is critical to increase research and knowledge about the disease at a cellular level. In this study, we sought to identify genes differentially regulated in diabetic type 1 and type 2 adipose-derived stem cells (ADSCs) and human aortic endothelial cells (HAECs). ADSCs are isolated from adipose tissue that can self-renew and are multipotent, immunoprivileged, and immunosuppressive. HAECs are endothelial cells that line macro blood vessels and play important roles in atherosclerosis, inflammation, barrier function, and angiogenesis. The primary cells are isolated from normal and diabetic type 1 and type 2 diagnosed human donors in accordance with all informed consent rules and regulations. The cells were cultured for several passages in optimized media. Gene expression analysis was performed using the Human Insulin Signaling 96 StellARray™ qPCR Array for ADSCs and the Human Endothelial Cell 96 StellARray™ qPCR Array for HAECs available from Bar Harbor Biotechnology. This article summarizes a study comparing normal and diabetic type 1 and type 2 ADSCs and HAECs to explore differences in gene expression among the donor samples.

Materials and Methods

In this study, we grew primary aortic endothelial and adipose-derived stem cells from normal, diabetic type 1 and diabetic type 2 tissues in standard submerged culture (see Table 1) to assess gene expression changes associated with diabetic diseased states.

	HAEC Aortic EC–Normal	HAEC Aortic EC–Diabetes Type 1	HAEC Aortic EC–Diabetes Type 2	Adipose-derived Stem Cells–Normal	Adipose-derived Stem Cells–Diabetes Type 1	Adipose-derived Stem Cells–Diabetes Type 2
Cat. No.	CC-2535	CC-2919	CC-2920	PT-5006	PT-5007	PT-5008
Lot No.	0000227953	0000239326	0000235247	7F4089	1F4104	1F4103
Age	49 Years	29 Years	53 Years	47 Years	62 Years	83 Years
Sex	Male	Female	Male	Female	Female	Female
Race	Asian	Caucasian	Caucasian	Unknown	Caucasian	Caucasian

Table 1. Donor characteristics of primary cells used.

Cell Isolation

Endothelial cells were isolated from normal and diabetic aortae. Isolated cells were expanded in standard submerged culture in EGM™ 2 Growth Medium (Lonza, cat. no. CC-3162) and then cryopreserved after the third passage. Adipose-derived stem cells from normal and diabetic donors were isolated and cryopreserved at the first passage in ADSC Growth Medium BulletKit™ (Lonza, cat. no. PT-4505). The vials were stored in liquid nitrogen until further use.

Cell Harvesting and Cell Lysis

Each lot of normal and diabetic endothelial cells was thawed and plated at a density of 5,000 cells/cm^2 in EGM™ 2 Growth Medium (Lonza, cat. no. CC-3162). Each lot of normal and diabetic adipose-derived stem cells was thawed and plated at a density of 5,000 cells/cm^2 in ADSC Growth Medium (Lonza, cat. no. PT-4505). Growth media were changed after 24 hours and the cells were subcultured through multiple passages. At P7, cells were pelleted and cell lysates were obtained using the QIAshredder™ column (Qiagen, cat. no. 79654).

qPCR Experimental Design

To generate data with biologically relevant variance, three replicate samples were independently assayed for each cell type.

RNA Isolation and cDNA Synthesis

Each cell lysate was transferred to the Qiagen RNeasy® Mini Kit (cat. no. 74104) and RNA was extracted. cDNA was synthesized with 2 µg of RNA per sample using SuperScript® II Reverse Transcriptase and dNTP mix (Life Technologies, cat. no. 18064-014 and 10297-018, respectively). For primers, random decamers and oligo dT primers (Life Technologies, Inc., cat. no. AM5722G and 18418-012) were used. cDNA synthesis reactions were performed according to the specifications of the supplier (see Table 2).

For Each qPCR Plate, a Reaction Mix was Prepared in the Following Manner	
2x SYBR® Green Master Mix (Life Technologies: Fast SYBR® Green Master Mix)	1031 µL
H_2O	975 µL
cDNA template: 40 µL cDNA synthesis reaction mix + 310 µL H_2O	106 µL

Table 2. Real-time qPCR.

20 µL of the reaction mix was distributed into Human Insulin Signaling 96 StellARray™ qPCR Array Plate for ADSCs and Human Endothelial Cell 96 StellARray™ qPCR Array Plate for HAECs. The master mixes contained AmpliTaq® Fast DNA Polymerase (Life Technologies, Inc.), designed to allow instant hot start. Arrays were run on the BioRad CFX 96 using a standard qPCR program. Post-run data collection involved the setting of a common threshold (Ct) across all arrays within an experiment, exportation and collation of the Ct values, and analysis via GPR.

Results and Discussion

The Human Insulin Signaling 96 StellARray™ qPCR Array was used to analyze the human adipose-derived stem cell samples. This array was designed with genes to measure the effects of insulin signaling associated with processes like glycogen synthesis, glycolysis, and fatty acid synthesis. The Human Endothelial Cell 96 StellARray™ qPCR Array was used to analyze the human aortic endothelial cell samples. This array was designed to measure genes associated with differentiation, development, and tissue remodeling of endothelium. In each cell type, statistically relevant differences in gene expression were detected in the diabetic cells compared to the normal cells. Genes with P-values of <0.05 or better are reported. Out of the 94 genes on the insulin array, the expression of 18 genes changed by +25.7 to -3.3 fold for ADSC type 1 diabetes and 10 genes changed by +24 to -7.7 fold for type 2 diabetes (as compared to normal cells). Out of the 94 genes on the endothelial array, the expression of 16 genes changed by +17 to -8.7 fold for HAEC type 1 diabetes and 8 genes changed by +52557 to -54 fold for type 2 diabetes (as compared to normal cells). While this article is not meant to be a comprehensive analysis of the genes and their functions in diabetes, we will highlight several genes and their functions.

ADSC: Adipose-derived Stem Cells

Among the ADSC diabetic type 1 and type 2 cells (Figures 1 and 2), the two most upregulated genes (as compared to the normal) for both were INS (insulin) and G6PC2 (glucose-6-phosphatase, catalytic, 2). G6PC2 encodes an enzyme that belongs to the G6PC family. These enzymes are part of a larger unit that catalyzes the hydrolysis of G6PC, which releases glucose into the bloodstream. The enzyme family member specific to G6PC2 is found in pancreatic islets and is a main target of cell-mediated autoimmunity in diabetes[1].

In the ADSC type 2 diabetic donors (Figure 2), PPARGC1A was down-regulated 7 fold as compared to the normal donors. PPARGC1A encodes for a transcriptional coactivator protein that regulates genes involved in energy metabolism[2].

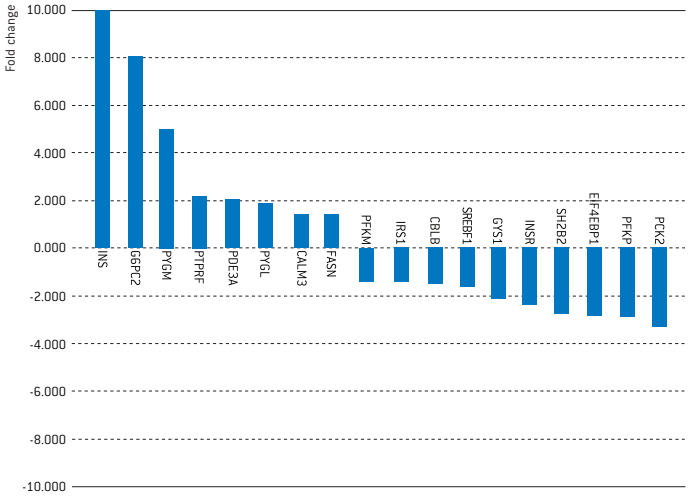

Figure 1. Genes up- or downregulated in ADSC diabetes type 1 sample vs. normal cells.

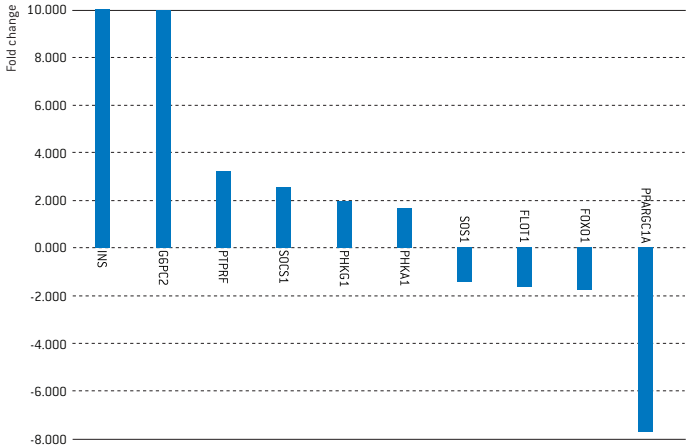

Figure 2. Genes up- or downregulated in ADSC diabetes type 2 sample vs. normal cells.

HAEC: Human Aortic Endothelial Cells

In the HAEC diabetic type 1 and type 2 cells (Figures 3 and 4), a common upregulated gene was SELE (14 fold in type 1 and 33.5 fold in type 2). E-selectin (SELE) is expressed by endothelial cells to recruit leukocytes during the inflammatory response, and increased levels of E-selectin are associated with type 2 diabetes. One study suggested that in high glucose conditions, macrophage stimulation of E-selectin may play a role in atherogenesis, and suggested this as a mechanism as to why arterial disease is accelerated in diabetes[3].

Hepatocyte growth factor (HGF) was downregulated in both type 1 (8.8 fold) and type 2 (54 fold) diabetic cells (Figures 3 and 4) when compared to the normal donor cells. Increasing the concentration of HGF has been shown to induce therapeutic angiogenesis in high glucose environments in rats and to lessen apoptosis in human endothelial cells[4,5]. Injury to aortic endothelial cells has been suggested to be a trigger of the progression of atherosclerosis in diabetic patients[5].

In the HAEC type 2 diabetic samples, AGER (advanced glycosylation end product-specific receptor) was downregulated 7 fold as compared to the normal donor samples. The receptor encoded by this gene interacts with molecules implicated in homeostasis, inflammation, and diabetes[6].

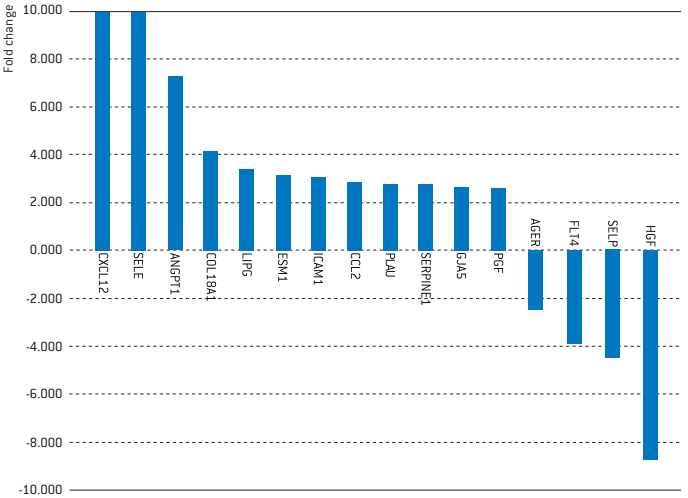

Figure 3. Genes up- or downregulated in HAEC diabetes type 1 sample vs. normal cells.

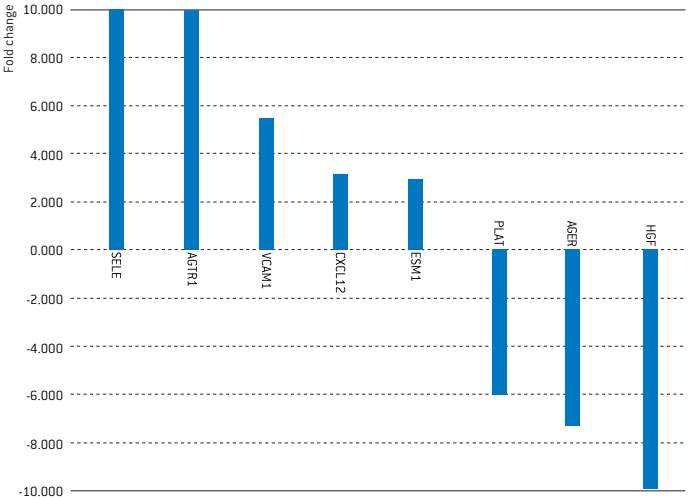

Figure 4. Genes up- or downregulated in HAEC diabetes type 2 sample vs. normal cells.

Summary

Data generated from this study shows up- and downregulation of genes in ADSC and HAEC cells from diabetic type 1 and type 2 donors as compared to normal donors using the StellARray™ qPCR Arrays. This data has indicated several differences in gene expression between the normal and diabetic cells from both cell types and is consistent with other published literature. The utility of primary cells has further strengthened the biological relevance of data generated as it relates more closely to the *in vivo* model. Results from this study encourage further exploration as to how diseases such as diabetes affect systems in the body and cause differences in gene expression and gene regulation pathways.

Lonza offers a variety of primary cells from diabetic type 1 and type 2 donors. In addition, Lonza introduced, through our Cells on Demand™ service, fresh pancreatic islets from normal and diabetic donors for research use. Type 1 diabetes is characterized by the immune system destroying insulin-secreting beta cells of the pancreas. Islet-transplantation replenishes the beta cell supply, rendering better regulation of insulin levels. Additional information about any of Lonza's cell offerings can be obtained from Lonza's Scientific Support Team.

References

1. Pubmed GENE ID 57818 description, (10Nov2012).
2. Pubmed GENE ID 10891 description, (10Nov2012).
3. Chen T, et al. *J Biol Chem.* 2011. 286(29):25564–73.
4. Taniyama Y, et al. *Circulation.* 2001. 104(19):2344–50.
5. Nakagami H, et al. *Diabetes.* 2002. 51(8):2604–2611.
6. Pubmed GENE ID 177 description, (10Nov2012).

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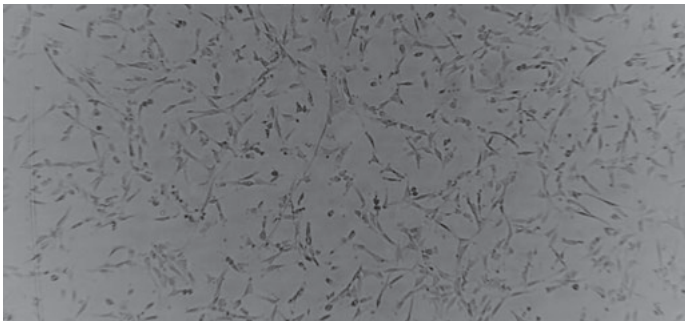

Figure 1. Image of human skeletal muscle cells (SkMC, CC-2561) cultured in SkGM using the CytoSMART™ Lux 10X System. Please refer to our website to view the video of a SkMC culture recorded with the CytoSMART™ System.

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Cat. No.	Description	Size
RAFT™ 3D Cell Culture System		
016-1R10	RAFT™ 96-well Bundle Kit, contains (016-0R94 & 016-0R92)	Kit
016-1R24	RAFT™ 24-well Bundle Kit, contains (016-0R94 & 016-1R32)	Kit
016-1R25	RAFT™ 24-well Insert Bundle Kit, contains (016-0R94 & 016-1R33)	Kit
016-1R16	RAFT™ Small Kit, 12 reactions, reagent and 24-well plate absorbers	Kit
016-1R32	RAFT™ Absorbers, for 24-well Plate	48
016-1R33	RAFT™ Insert Absorbers, for 24-well Plate,	48 inserts
016-0R92	RAFT™ Plate Kit, 4 × 96-well clear plate and 4 × 96-well absorber	Kit
016-0R94	RAFT™ Reagent Kit, Reagent Kit for 3D Cell Cultures	Kit
CytoSMART™ System		
AACS-1001	CytoSMART™ LUX 10X System, contains CytoSMART™ Lux 10X Device, accompanying tablet and two year free CytoSMART™ Connect Cloud Service	–
AWCS-1001	CytoSMART™ Connect Cloud One Year Renewal, Automatic renewal of CytoSMART™ Connect Cloud Service for one year	–
AAK-2003	CytoSMART™ Stage Attachment, allows viewing of T162 flasks	–
Cardiovascular Cells		
CC-2535	HAEC – Human Aortic Endothelial Cells in EGM™ 2, cryopreserved	≥500 000 cells
CC-2920	D-HAEC – Human Aortic endo, Diabetes Type II in EGM™ 2, cryopreserved	≥500 000 cells
CC-2919	D-HAEC – Human Aortic endo, Diabetes Type I in EGM™ 2, cryopreserved	≥500 000 cells
CC-2585	HCAEC – Human Coronary Artery Endothelial Cells in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2922	D-HCAEC – Human Coronary Artery Endothelial Cells, Diabetes Type II in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2921	D-HCAEC – Human Coronary Artery Endothelial Cells, Diabetes Type I in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2530	HPAEC – Human Pulmonary Artery Endothelial Cells in EGM™ 2, cryopreserved	≥500 000 cells
CC-2924	D-HPAEC – Human Pulmonary Artery Endothelial Cells, Diabetes Type II in EGM™ 2, cryopreserved	≥500 000 cells
CC-2923	D-HPAEC – Human Pulmonary Artery Endothelial Cells, Diabetes Type I in EGM™ 2, cryopreserved	≥500 000 cells
CC-7030	HMVEC-C – Human Cardiac Microvascular Endothelial Cells in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2928	D-HMVEC – Human Cardiac Microvascular Endothelial Cells, Diabetes Type II in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2927	D-HMVEC – Human Cardiac Microvascular Endothelial Cells, Diabetes Type I in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2543	HMVEC-dAd – Human Adult Dermal Microvascular Endothelial Cells in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2930	D-HMVEC – Human Dermal Microvascular Endothelial Cells, Diabetes Type II in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2929	D-HMVEC – Human Dermal Microvascular Endothelial Cells, Diabetes Type I in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2545	HIAEC – Human Iliac Artery Endothelial Cells in EGM™ 2MV, cryopreserved	≥500 000 cells
C2519A	HUVEC – Human Umbilical Vein Endothelial Cells, pooled, in EGM™ 2, cryopreserved	≥500 000 cells
C2517A	HUVEC – Human Umbilical Vein Endothelial Cells, single donor, in EGM™ 2, cryopreserved	≥500 000 cells
00191027	HUVEC-XL™ – Human Umbilical Vein Endothelial Cells, expanded, cryopreserved	≥10 million cells
CC-2527	HMVEC-L – Human Lung Microvascular Endothelial Cells in EGM™ 2MV, cryopreserved	≥500 000 cells
CC-2581	PASMC – Human Pulmonary Artery Smooth Muscle Cells, in SmGM™ 2, cryopreserved	≥500 000 cells
CC-2913	D-PASMC – Human Pulmonary Artery Smooth Muscle Cells, Diabetes Type II in SmGM™ 2, cryopreserved	≥500 000 cells
CC-2915	D-PASMC – Human Pulmonary Artery Smooth Muscle Cells, Diabetes Type I in SmGM™ 2, cryopreserved	≥500 000 cells
CC-2571	AoSMC – Human Aortic Smooth Muscle Cells, in SmGM™ 2, cryopreserved	≥500 000 cells
CC-2916	D-AoSMC – Human Aortic Smooth Muscle Cells, Diabetes Type II in SmGM™ 2, cryopreserved	≥500 000 cells
CC-2914	D-AoSMC – Human Aortic Smooth Muscle Cells, Diabetes Type I in SmGM™ 2, cryopreserved	≥500 000 cells
CC-2583	CASMC – Human Coronary Artery Smooth Muscle Cells, in SmGM™ 2, cryopreserved	≥500 000 cells

Cat. No.	Description	Size
Cardiovascular Cells (Continued)		
CC-2918	D-CASMC – Human Coronary Artery Smooth Muscle Cells, Diabetes Type II in SmGM™ 2, cryopreserved	≥500 000 cells
CC-2917	D-CASMC – Human Coronary Artery Smooth Muscle Cells, Diabetes Type I in SmGM™ 2, cryopreserved	≥500 000 cells
CC-7014	AoAF – Human Aortic Adventitial Fibroblasts in SCGM™, cryopreserved	≥500 000 cells
CC-2579	UASMC – Human Umbilical Artery Smooth Muscle Cells, in SmGM™ 2, cryopreserved	≥500 000 cells
Skeletal Muscle Cells		
CC-2580	HSMM – Human Skeletal Muscle Myoblasts, in SkGM™ 2, cryopreserved	≥500 000 cells
CC-2901	D-HSMM – Human Skeletal Muscle Myoblasts, Diabetes Type II in SkGM™ 2, cryopreserved	≥500 000 cells
CC-2900	D-HSMM – Human Skeletal Muscle Myoblasts, Diabetes Type I in SkGM™ 2, cryopreserved	≥500 000 cells
Intestinal Cells		
CC-2902	H-InMyoFib – Human Intestinal Myofibroblasts in SmGM™, cryopreserved	≥500 000 cells
CC-2931	H-InEpC – Human Intestinal Epithelial Cells in SmGM™, cryopreserved	≥800 000 cells
Renal Cells		
CC-2553	RPTEC – Human Renal Proximal Tubule Cells in REGM™, cryopreserved	≥500 000 cells
CC-2925	D-RPTEC – Human Renal Proximal Tubule Cells, Diabetes Type II in REGM™, cryopreserved	≥500 000 cells
CC-2554	HRCE – Human Renal Cortical Epithelial Cells in REGM™, cryopreserved	≥500 000 cells
CC-2556	HRE – Human Renal Epithelial Cells in REGM™, cryopreserved	≥500 000 cells
Dermal Cells		
00192627	HRE – Human Renal Epithelial Cells in REGM, in KGM™ Gold, cryopreserved	≥500 000 cells
CC-2926	D-HEK – Human Adult Epidermal Keratinocytes, Diabetes Type II in KGM™ Gold, cryopreserved	≥500 000 cells
Adipose Cells		
PT-5005	Preadipocytes, Human Visceral Cells in PGM™ 2, cryopreserved	≥1 million cells
PT-5024	Preadipocytes, Human Visceral Cells, Diabetes Type II in PGM™ 2, cryopreserved	≥1 million cells
PT-5023	Preadipocytes, Human Visceral Cells, Diabetes Type I in PGM™ 2, cryopreserved	≥1 million cells
PT-5020	Preadipocytes, Human Subcutaneous Cells in PGM™ 2, cryopreserved	≥1 million cells
PT-5001	Preadipocytes, Human Subcutaneous Cells in PGM™ 2, cryopreserved	≥4 million cells
PT-5022	Preadipocytes, Human Subcutaneous Cells, Diabetes Type II in PGM™ 2, cryopreserved	≥1 million cells
PT-5021	Preadipocytes, Human Subcutaneous Cells Diabetes Type I in PGM™ 2, cryopreserved	≥1 million cells
PT-5006	Adipose-Derived Human Stem Cells	≥1 million cells
PT-5008	D-ADSC – Adipose-Derived Human Stem Cells, Diabetes Type II	≥1 million cells
PT-5007	D-ADSC – Adipose-Derived Human Stem Cells, Diabetes Type I	≥1 million cells

Metabolic Research – Related Media on next page.

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Ordering Information

Cat. No.	Description	Size
Immortalized Cells for Metabolic Research		
XM13A1	Skeletal Muscle Cells	—
XM15B1	Skeletal Muscle Cells	—
XA15A1	Adipocytes	—
XF05C1	Human Dermal Fibroblasts	—
XS12C1	Human Coronary Artery Smooth Muscle Cells	—
XSEL6C1	Dermal Microvascular LEC	—
XSEB113C1	Dermal Microvascular BEC	—
XSKA1B1	Adult Keratinocytes	—
00194607	Human Brain Microvascular Endothelial Cells	—
Metabolic Research Media		
CC-3162	EGM™ 2 – Endothelial Cell Growth Medium-2 BulletKit™	Kit
CC-3202	EGM™ 2MV – Microvascular Endothelial Cell Growth Medium-2 BulletKit™	Kit
00192060	KGM™ Gold – Keratinocyte Growth Medium BulletKit™	Kit
CC-3190	REGM™ – Renal Epithelial Cell Growth Medium BulletKit™	Kit
CC-3245	SkGM™ 2 – Skeletal Muscle Cell Growth Medium-2 BulletKit™	Kit
CC-3182	SmGM™ 2 – Smooth Muscle Growth Medium-2 BulletKit™	Kit
PT-8002	PGM™ 2 – Preadipocyte Growth Medium-2 BulletKit™	Kit
PT-4505	ADSC Growth Medium BulletKit™	Kit
CC-5034	ReagentPack™ – Subculture Reagents	100 mL each
Metabolic Research-Related Assays		
00193339	AdipoLyze™ Lipolysis Detection Assay	1 × 96-wells
PT-7009	AdipoRed™ Adipogenesis Assay Reagent	5 × 4.0 mL
Nucleofector™ Devices		
AAB-1001	Nucleofector™ 2b Device	—
AAF-1001B	4D-Nucleofector™ Core Unit	—
AAF-1001X	4D-Nucleofector™ X Unit	—
AAM-1001S	96-well Shuttle™ Device	—
Kits for 4D-Nucleofector™		
V4XP-1012	P1 Primary Cell Kit	12 rxn (100 µl Nucleocuvette™)
V4XP-1024		24 rxn (100 µl Nucleocuvette™)
V4XP-1032		32 rxn (20 µl Nucleocuvette™; 16-well)
V4XP-2012	P2 Primary Cell Kit	12 rxn (100 µl Nucleocuvette™)
V4XP-2024		24 rxn (100 µl Nucleocuvette™)
V4XP-2032		32 rxn (20 µl Nucleocuvette™; 16-well)
V4XP-3012	P3 Primary Cell Kit	12 rxn (100 µl Nucleocuvette™)
V4XP-3024		24 rxn (100 µl Nucleocuvette™)
V4XP-3032		32 rxn (20 µl Nucleocuvette™; 16-well)
V4XP-4012	P4 Primary Cell Kit	12 rxn (100 µl Nucleocuvette™)
V4XP-4024		24 rxn (100 µl Nucleocuvette™)
V4XP-4032		32 rxn (20 µl Nucleocuvette™; 16-well)

Cat. No.	Description	Size
Kits for 4D-Nucleofector™ (Continued)		
V4XP-5012	P5 Primary Cell Kit	12 rxn (100 µl Nucleocuvette™)
V4XP-5024		24 rxn (100 µl Nucleocuvette™)
V4XP-5032		32 rxn (20 µl Nucleocuvette™; 16-well)
V4XP-9096	Primary Cell Optimization Kit	96 rxn (20 µl Nucleocuvette™; 16-well)
V4XC-1012	SE Cell Line Kit	12 rxn (100 µl Nucleocuvette™)
V4XC-1024		24 rxn (100 µl Nucleocuvette™)
V4XC-1032		32 rxn (20 µl Nucleocuvette™; 16-well)
V4XC-2012	SF Cell Line Kit	12 rxn (100 µl Nucleocuvette™)
V4XC-2024		24 rxn (100 µl Nucleocuvette™)
V4XC-2032		32 rxn (20 µl Nucleocuvette™; 16-well)
V4XC-3012	SG Cell Line Kit	12 rxn (100 µl Nucleocuvette™)
V4XC-3024		24 rxn (100 µl Nucleocuvette™)
V4XC-3032		32 rxn (20 µl Nucleocuvette™; 16-well)
V4XC-9064	Cell Line Optimization Kit	64 rxn (20 µl Nucleocuvette™; 16-well)
Kits for 96-well Shuttle™ Device		
V4SP-1096	P1 Primary Cell Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-1960		960 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-2096	P2 Primary Cell Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-2960		960 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-3096	P3 Primary Cell Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-3960		960 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-4096	P4 Primary Cell Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-4960		960 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-5096	P5 Primary Cell Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-5960		960 rxn (20 µl Nucleocuvette™; 96-well)
V4SP-9096	Primary Cell Optimization Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SC-1096	SE Cell Line Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SC-1960		960 rxn (20 µl Nucleocuvette™; 96-well)
V4SC-2096	SF Cell Line Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SC-2960		960 rxn (20 µl Nucleocuvette™; 96-well)
V4SC-3096	SG Cell Line Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
V4SC-3960		960 rxn (20 µl Nucleocuvette™; 96-well)
V4SC-9096	Cell Line Optimization 96-Well-Nucleofector™ Kit	96 rxn (20 µl Nucleocuvette™; 96-well)
Kits for Nucleofector™ II/2b Device		
VAPC-1001	AoSMC Nucleofector™ Kit	10 rxn (100 µL aluminum cuvette)
VPC-1001		25 rxn (100 µL aluminum cuvette)
VVPC-1001		4 × 25 rxn (100 µL aluminum cuvette)
VAPI-1004	Basic Smooth Muscle Cell Nucleofector™ Kit	10 rxn (100 µL aluminum cuvette)
VPI-1004		25 rxn (100 µL aluminum cuvette)
VVPI-1004		4 × 25 rxn (100 µL aluminum cuvette)

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Ordering Information

Cat. No.	Description	Size
Kits for Nucleofector™ II/2b Device (Continued)		
VAPB-1001	HCAEC Nucloefector™ Kit	10 rxn (100 µL aluminum cuvette)
VPB-1001		25 rxn (100 µL aluminum cuvette)
VVPB-1001		4 × 25 rxn (100 µL aluminum cuvette)
VAPI-1001	Basic Endothelial Cell Nucleofector™ Kit	10 rxn (100 µL aluminum cuvette)
VPI-1001		25 rxn (100 µL aluminum cuvette)
VVPI-1001		4 × 25 rxn (100 µL aluminum cuvette)
VAPB-1002	HUVEC Nucleofector™ Kit	10 rxn (100 µL aluminum cuvette)
VPB-1002		25 rxn (100 µL aluminum cuvette)
VVPB-1002		4 × 25 rxn (100 µL aluminum cuvette)
VAPB-1003	HMVEC-L Nucloefector™ Kit	10 rxn (100 µL aluminum cuvette)
VPB-1003		25 rxn (100 µL aluminum cuvette)
VVPB-1003		4 × 25 rxn (100 µL aluminum cuvette)
VAPI-1002	Basic Fibroblast Nucloefector™ Kit	10 rxn (100 µL aluminum cuvette)
VPI-1002		25 rxn (100 µL aluminum cuvette)
VVPI-1002		4 × 25 rxn (100 µL aluminum cuvette)
VAPI-1005	Basic Epithelial cell Nucleofector™ Kit	10 rxn (100 µL aluminum cuvette)
VPI-1005		25 rxn (100 µL aluminum cuvette)
VVPI-1005		4 × 25 rxn (100 µL aluminum cuvette)
VAPD-1002	Human Keratinocyte Nucleofector™ Kit	10 rxn (100 µL aluminum cuvette)
VPD-1002		25 rxn (100 µL aluminum cuvette)
VVPD-1002		4 × 25 rxn (100 µL aluminum cuvette)
VAPE-1001	Human MSC Nucleofector™ Kit	10 rxn (100 µL aluminum cuvette)
VPE-1001		25 rxn (100 µL aluminum cuvette)
VVPE-1001		4 × 25 rxn (100 µL aluminum cuvette)
VAPL-1004	Mouse/Rat Hepatocyte Nucleofector™ Kit	10 rxn (100 µL aluminum cuvette)
VPL-1004		25 rxn (100 µL aluminum cuvette)
VVPL-1004		4 × 25 rxn (100 µL aluminum cuvette)
VACA-1005	Cell Line L	10 rxn (100 µL aluminum cuvette)
VCA-1005		25 rxn (100 µL aluminum cuvette)
VVCA-1005		4 × 25 rxn (100 µL aluminum cuvette)
VACA-1002	Cell Line T	10 rxn (100 µL aluminum cuvette)
VCA-1002		25 rxn (100 µL aluminum cuvette)
VVCA-1002		4 × 25 rxn (100 µL aluminum cuvette)
VACA-1003	Cell Line V	10 rxn (100 µL aluminum cuvette)
VCA-1003		25 rxn (100 µL aluminum cuvette)
VVCA-1003		4 × 25 rxn (100 µL aluminum cuvette)
VACA-1001	Cell Line R	10 rxn (100 µL aluminum cuvette)
VCA-1001		25 rxn (100 µL aluminum cuvette)
VVCA-1001		4 × 25 rxn (100 µL aluminum cuvette)
VACA-1004	Cell Line C	10 rxn (100 µL aluminum cuvette)
VCA-1004		25 rxn (100 µL aluminum cuvette)
VVCA-1004		4 × 25 rxn (100 µL aluminum cuvette)
VCO-1001N	Cell Line Optimization Kit	18 reactions (100 µL aluminum cuvette)

Cat. No.	Description	Size
FlashGel™ System for RNA		
57027	FlashGel™ RNA Cassettes	1.2% agarose, 12 + 1 single-tier, 9 pk
57028		1.2% agarose, 16 + 1 double-tier (34-well), 9 pk
50462	FlashGel™ Loading Dye	5 × 1 mL, 5X concentration, RNA native sample buffer
50577	FlashGel™ RNA Marker	50 µg, band sizes: 0.5/1/1.5/3/5/9 kb
57024	FlashGel™ RNA Starter Kit	Includes: 9 pk FlashGel™ RNA Cassettes (1.2% agarose, 12 + 1 well); Formaldehyde sample buffer; FlashGel™ RNA Marker; and AccuGENE™ Molecular Biology Water.

PAGEr™ Gels and Accessories

Gel Concentration/Separation Range	Cassette Size (cm)	Cat. No. 2D-Well	Cat. No. 10-Well	Cat. No. 12-Well	Cat. No. 16-Well	Cat. No. 17-Well	Cat. No. 8 + 1 Well
4 – 12% gradient	9 × 10	–	58520	58522	58524	–	–
25 – 250 kDa	10 × 10	–	59520	59522	59524	–	–
4 – 20% gradient	9 × 10	–	58511	58505	58517	58545	58551
5 – 200 kDa	10 × 10	59557	59511	59505	59517	59545	59551
8 – 16% gradient	9 × 10	–	58519	58521	58523	58560	58562
15 – 200 kDa	10 × 10	59564	59519	59521	59523	59560	59562
10 – 20% gradient	9 × 10	–	58512	58506	58518	–	–
5 – 150 kDa	10 × 10	–	59512	59506	59518	–	–
7.5%	9 × 10	–	58507	58501	58513	58540	–
50 – 200 kDa	10 × 10	–	59507	59501	59513	–	–
10%	9 × 10	–	58508	58502	58514	58542	58548
25 – 200 kDa	10 × 10	59554	59508	59502	59514	59542	59548
12%	9 × 10	–	58509	58503	58515	58543	–
20 – 200 kDa	10 × 10	59571	59509	59503	59515	59543	–
15%	9 × 10	–	58510	58504	58516	58544	58550
10 – 50 kDa	10 × 10	59556	59510	59504	59516	59544	59550

www.lonza.com/protein – For PAGEr™ Gel formats and accessories.

www.lonza.com/research
www.lonza.com/metabolic

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