Primary Human Cells from Life Technologies- Offerings, Scale and Applications in HCS and HTS Formats

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ABSTRACT

Normal human primary cells in culture provide physiologically relevant cell systems for the analysis of complex biological processes and pathway dissection. Unlike commonly used immortalized cell lines, normal human primary cells retain many of the characteristics of the tissues from which they were derived. Thus, primary cells provide more appropriate models of human biology, which is critical in enabling effective basic research and drug development. Several practical concerns have limited wider adoption of primary cells in pharmaceutical research, including scale, donor-to-donor consistency, ease of use and efficient methods of content delivery for assays and analysis.

Here we address several of these issues, providing information on typical lot sizes and cell provisioning capabilities (scale) based on minimum guaranteed cell growth potential. Workflow simplifications increasing ease of use are outlined for our new and unique rapid human skeletal myoblast (HSkM) differentiation system, in which >50% of cells form multinucleated scincitia 48 hr after seeding into plates ranging between 6-well to 384-well. Relevance to human muscle biology is underscored by results showing incubation with physiologically levels of TGFb blocked differentiation, while this effect was blunted by addition of IGF-I (5 nM). Similar effects were seen using Myostatin, a known negative regulator of muscle mass. Parallel BacMam (recombinant baculovirus encoding exogenous proteins of interest whose expression driven by a mammalian promoter) enabled LanthaScreen® TR-FRET (time resolved fluorescent energy transfer) studies interrogated the effects of these growth factors on their cognate signaling pathways—PI3K-Akt (using PRAS40-GFP phosphorylation) and TGFβ-Smad (using Smad3-GFP phosphorylation). Additional electrophysiological studies of endogenous voltage gated calcium currents in the HSkM using the Fluo-4 Direct™ assay demonstrated dose-dependent Verapimil and Nifedipine inhibition following KCl depolarization .

Image based studies incorporated a range of different primary cells types including human corneal epithelial cells, dermal fibroblasts and epidermal keratinocytes. Multiple assays were undertaken including cell proliferation, autophagy and dynamic cytoskeletal reorganization following incubation with various compounds including Chloroquine, Cytochalsin D and Nocodazole. Imaging of these HTS compatible assays was enabled using numerous cell labeling tools from Life Technologies including, organic dyes, Alexa Fluor® labeled antibodies and Click-iT® metabolic labeling and detection reagents. Taken together our results underscore the broad utility of pairing normal human primary cells with assay and imaging tools from Life Technologies for use in different drug discovery paradigms enabling interrogation of targets or pathways of interest in physiologically relevant cell systems.

MATERIALS AND METHODS

Cell Culture- All normal human primary cells were from Life Technologies. Cells were cultured under standard conditions as recommended in the product instructions. **BacMam-** BacMam 2.0 transductions were carried out in complete media, incubated over night using standard culture conditions (37C/5%CO_2) using indicated particle concentrations. BacMam enabled LanthaScreen TR-FRET assays were delivered to myofibers following 48 hrs in DM, and indicated assays were performed the next day. **Myogenic index-**(% of cells forming multinucleated structures) was determined by quantitative immunofluorescent staining for Troponin. **Fluo-4 Direct™ assays-** were performed as described and imaged using Hamamatsu FDSS. **Antibody and fluorescent phalloidin** incubations were performed in DPBS supplemented with 5% NGS and 3% BSA, anti LC3B (0.5 ug/ml) and anti-tubulin (2.0 ug/ml). Alexa Fluor® phalloidin (1:300) for cell staining. **Click-iT® EdU** incorporation was carried out in complete media for 1 hour at 37C at a final concentration of 10μM EdU. EdU detection was carried out via click chemistry using azide-modified Alexa Fluor® 488 dye

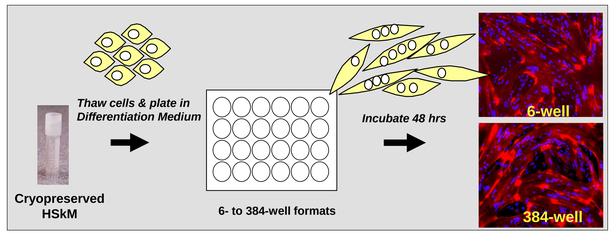
RESULTS

Table 1. Selected Primary Cell Types, Lot Sizes and Expansion Potential

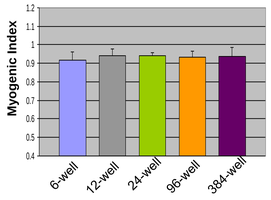
Cell Type	Ave lot size (vials)	Minimum lifespan (population doublings*)	Expansion Capability (per vial**)	Potential 384-well (plates/vial***)
HEKn (Human Epidermal Keratinocytes-neonatal)	100	≥ 30 PDs	2.7 x10^{14} cells	7 x10^{7} plates
HDFn (Human Dermal Fibroblasts-neonatal)	50	≥ 25 PDs	8.4 x10^{12} cells	2 x10^{6} plates
HEMn (Human Epidermal Melanocytes-neonatal)	135	≥ 16 PDs	1.6 x10^{10} cells	4 x10^{4} plates
HUVEC (Human Umbilical Vein Endothelial Cells)	60	≥ 16 PDs	1.6 x10^{10} cells	4 x10^{4} plates
HCEC (Human Corneal Epithelial Cells	125	≥ 12 PDs	1.0 x10^{9} cells	260 plates
HSkM (Human Skeletal Myoblasts)	250	NA	NA	1-2 plates
HASMC (Human Aortic Smooth Muscle Cells)	90	≥ 16 PDs	1.6 x10^{10} cells	4 x10^{4} plates

* Typically 3-4 population doublings occur per passage
**Expansion estimates based on expanding 50% of minimum viable cells/vial through minimum long term growth potential
Expansion calculations: $N_t = N_o 2^x$ where N_t = # of cells at Time t, N_o= # of cells at Time o, and x= population doublings
*** Assumes 10,000 cells/well in 384 well plate

Figure 1. Human Skeletal Myoblast (HSkM) Rapid Differentiation System

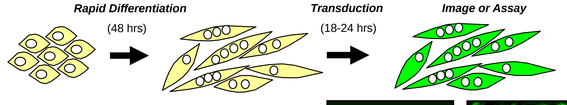


Consistent Differentiation Across a Range of Multi-well Formats



- **Easy to use system**
- **Validated for differentiation capacity: >50%**
- **No specialty medium (DMEM and Horse Serum) or cell counting required**
- **Large lot size from single donors available**
- **No medium changes or tissue culture vessel coatings required**

Figure 2. High efficiency delivery of BacMam2.0 encoded fluorescent proteins in differentiated myofibers



- **Differentiated myofibers incubated with BacMam encoding GFP diluted in DM**
- **Cells fixed with 4% PFA, stained and imaged**
- **>80% of myofibers expressed GFP**

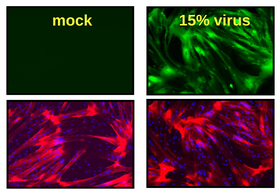


Figure 3. Interrogation of IGF and TGFβ Pathways using in BacMam-enabled LanthaScreen® Cellular Assays in Differentiated Myofibers

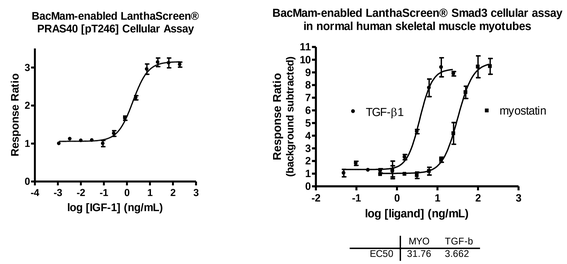
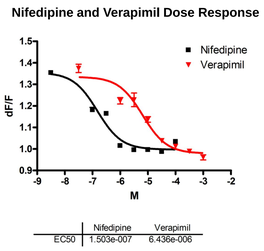


Figure 4. Endogenous Voltage Gated Calcium Currents in HSkM using the Fluo-4 Direct™ Assay



- **HSkM plated in 384 well microplates and incubated 48 hr in differentiation medium**
- **Fluo4 Direct™ reagent (1x) was diluted in HBSS + 20 mM HEPES with indicated concentrations of Nifedipine or Verapimil**
- **Cells were incubated for 60 min at 37C as indicated**
- **Stimulation with 30 mM KCl depolariztion**

Figure 5. Image Based Quantification of Chloroquine (CQ)-induced Autophagy in Normal Human Primary Cells

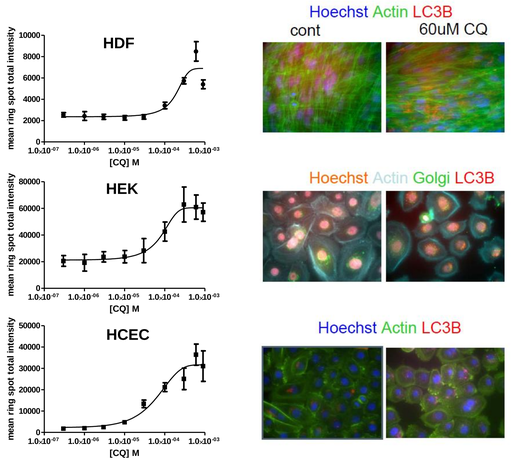
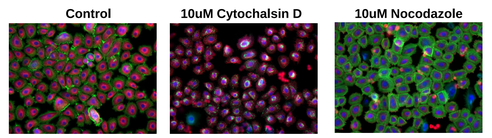


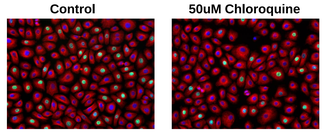
Figure 6. Multi-parametric Fluorescent Imaging of Normal Human Corneal Epithelial Cells

Visualizing impacts of Microfilament Disruption in HCEC



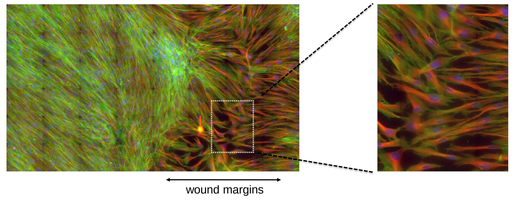
Imaging Reagents: CellMask™ Blue stain (*blue- nuclei*) /Alexa Fluor® 488 Phalloidin (*green- actin*)/ Anti α tubulin-Goat & anti- mouse Alexa Fluor® 555 (*red- tubulin*)

Chloroquine induced reduction in DNA synthesis in HCEC



Imaging Reagents: CellMask™ Blue stain (*blue- nuclei*) /Alexa Fluor® 488 Click-iT® EdU (*green- DNA synthesis)*/Anti α tubulin-Goat anti mouse Alexa Fluor® 555 (*red- tubulin*)

Figure 7. Fluorescent Imaging of Normal Human Dermal Fibroblasts Following Scratch Wound



Imaging Reagents: CellMask™ Blue stain (*blue- nuclei*) /Alexa Fluor® 488 Phalloidin (*green- actin*)/ Anti α tubulin-Goat & anti- mouse Alexa Fluor® 555 (*red- tubulin*)

SUMMARY

- **Life Technologies offers a broad range of normal human primary cell systems reagents and imaging tools which support drug discovery workflows**
- **Normal human primary cells provide physiologically relevant systems for modeling human biology**
- **BacMam Technology from Life Technologies (including CellLight® Reagents and LanthaScreen® GFP fusion proteins) enable assay portability and are compatible with HTS and HCS workflows and formats**