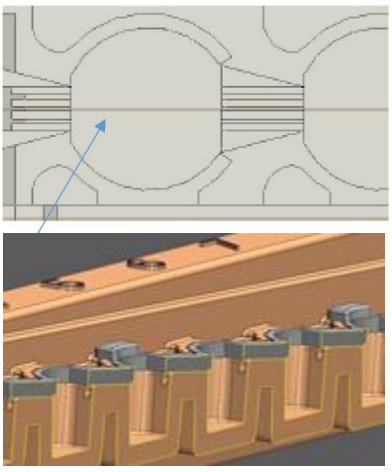
Basic Protocol for the SciFlow™1000 System
CellTitre-Glo® Assay to Measure ATP Content
Live Cell Numbers (end-study assay)
Monolayer Culture (media signal)

System Description:

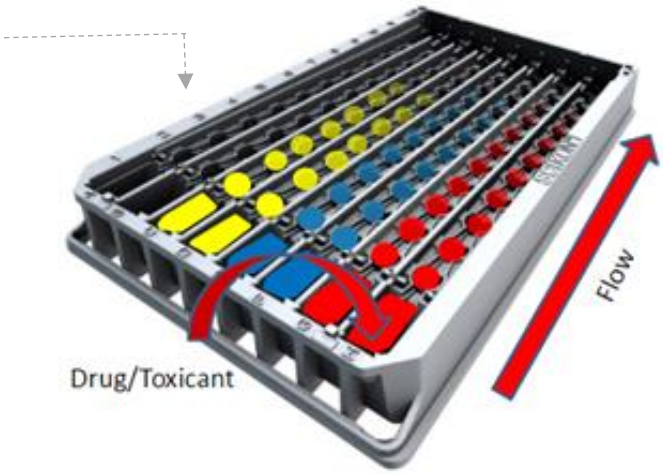
The SciFlow™ 1000 Fluidic Culture System is a benchtop tool for *in vitro* use to mimic cell, organ, and living systems. SciFlow operates like a shallow river bed with a series of compartments for cell culture. The design allows for isolated and stagnant culture during cell seeding then delivers real-time fluid flow and compartment-to-compartment signaling over time. The entire system is contained within a 96-well formatted culture plate that includes 8 repeatable channels. Each channel has the capacity to connect 1-to-10 cell culture wells in a linear array. As a benchtop tool, SciFlow is configured for cell and tissue assessments allowing easy access to all culture wells and media streams. Additionally, SciFlow is compatible with microplate readers, high content imaging platforms, and microscopes. Beneficially, no external pumps, or tubes, or controllers are required.

Protocol Focus: There are many applications of the SciFlow 1000, this protocol focuses on measuring the ATP content of cells, referencing adherent type cells. It is used to determine the number of live cells in culture, in each of the 9 interconnected cellular compartments of the SciFlow system.

Overview: Culture areas within SciFlow 1000 are analogous to a ½ area 96-well plate (0.167 cm^2 per well). As purchased, all culture wells are tissue culture treated.



Surface & profile images of linear aligned culture wells



Background: The CellTitre-Glo 2.0® Assay is a method for determining the number of viable cells in culture by measuring the amount of ATP present, which indicates the presence of metabolically active cells. The amount of ATP is directly proportional to the number of cells present in culture. The CellTitre-Glo 2.0® assay generates a "glow-type" luminescent signal, which has a half-life generally greater than three hours, depending on cell type and medium used. The extended half-life eliminates the need to use reagent injectors and provides flexibility for continuous or batch-mode processing of multiple plates.

This protocol calls for inverting the plate to remove all media and to stop flow. It is recommended for adherent cells. CellTitre-Glo®r results in cell lysis, therefore this is an end-study assay.

Materials: CellTitre-Glo® (Promega, catalog number G9242)

Reagent Stability and Storage: CellTitre-Glo® is shipped as a single reagent at -20C. When ready to be used, the reagent is thawed at 4C (overnight). It can be stored at 4C without loss of activity for up to 5 months. Prior to use in the assay allow the reagent to warm to room temperature. **Do not warm in water bath.** Mix gently by inversion prior to use.

CellTitre-Glo® Manufactures Protocol:
http://www.promega.com/~/media/files/resources/protocols/technical%20manuals/101/celltiterglo%202%200%20assay%20protocol.pdf

Equipment:

SciFlow 1000 System
Multichannel pipette
Pipette Tips
Greiner while solid bottom 96-well half area plate
Plate reader with luminometer capabilities

Standard Curve/Correlations:

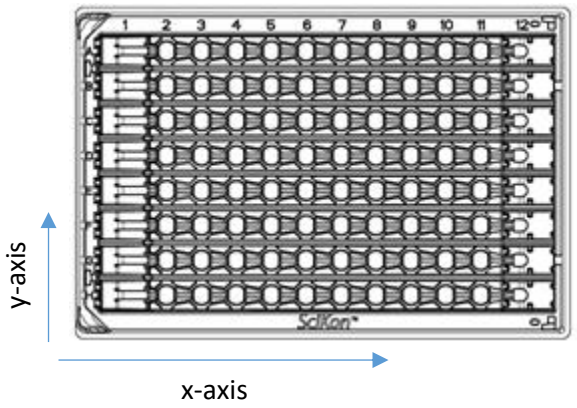
Data can be normalized against control cultures, such as vehicle only cultures within the same SciFlow system.

Protocol:

Dilution of CellTitre-Glo: CellTitre-Glo® is manufactured as a 2X solution and is meant to be used by diluting into equal volumes of cell culture media in the tissue culture plate. The SciFlow 1000 system has been engineered to achieve flow between wells, but that may not be desirable when performing this assay. This protocol removes all media from the well, then adds 1X CellTitre-Glo to the empty well to eliminate flow of the CellTitre-Glo® reagent, between wells, during the assay.

1. Determine the amount of CellTitre-Glo® reagent required
    a. For the SciFlow system, use 20 µL /well. Example: There are 54 wells, containing cells, in a SciFlow 1000 System (well 3 – 11 using 6 rows; 2 rows are acellular). Round this number to 60 wells. 60wells x 20 µL /well = 1200 µLs of CellTitre-Glo®. Add an equal volume of 1x PBS into a 15ml tube. Total of 2400 uls.
2. Remove as much media as possible from SciFlow.
    a. SciFlow can be emptied by inversion and flicking into an appropriate waste container. Flick the system over the sink or onto paper towels, then invert the system on a paper towel for 90 seconds to remove all the media. Alternatively, vacuum suction the media out.
3. Add 40 µL of the diluted CellTitre-Glo® reagent to each well of the SciFlow 1000 System
4. Incubate at room temperature for 10 minutes. Keep the system as flat as possible to avoid flow.
5. Transfer 20 µL s from each well to the corresponding well of a Greiner white ½ well 96-well plate for use in the luminometer.
    a. *Note*: Tilt the SciFlow System towards you along the shorter axis (y-axis) to help pool the solution. Do not tilt SciFlow along the lengthy axis (x-axis) as this can disrupt or modify flow and any established signals. Pipette slowly to reduce bubbles. A manual multichannel pipette is recommended for this step as it may allow increased control over aspiration.

SciFlow 1000 System



6. Read the plate in a luminometer.

Tips and FAQ: This section outlines some very useful techniques for handling SciFlow 1000.

Removing Media (if required): SciFlow can be emptied by inversion and flicking into an appropriate waste container. Additionally, the entire row can be emptied via vacuum aspiration through the sink well.

Adding Media (if required): When adding fluid to SciFlow, it is best to begin additions at the lower end of the plate (Column 11), followed by columns 10 then 9…. This will result in downstream wells being filled before the upstream wells are filled, and result in a much more controlled fluid flow.

Cell Number: The SciFlow 1000 is a ½ area 96-well plate, the number of cells seeded in each well should be adjusted accordingly. See cell seeding table at the end of this protocol.

Compound Addition: When initiating a gradient, the most reproducible method is to add a small volume of a more concentrated stock solution into the source well. When adding the more concentrated stock, do not pipette up and down (to mix) as this will change the flow dynamics. Add gently.

Feeding/Dosing: For experiments requiring incubations longer than 24 hours or for repeated exposures to the compounds of interest, additional media/compound must be added to the source well. Volumes between 50µl – 250µl can be used for dosing/feeding, in most cases 150µl is an appropriate volume. Whatever volume is chosen, the same volume should be initially removed from column 12 (waste) and a replicate volume of fresh media or 1x compound in fresh media added into the source well of the SciFlow 1000.

Tracking Flow: Fluorescein can be used as a tracking dye to monitor the flow during an experiment and to approximate compound concentrations in each well, not accounting for cellular metabolism. See Application Note for detailed instructions on quantifying fluorescein and compound concentrations.

Moving SciFlow 1000: SciFlow is a fluidic system and if the plate is tipped along the lengthy axis (x-axis), this can disrupt or modify both flow and any established gradients. Reasonable care should be taken when moving the plate to minimize unintended flow caused by tipping the plate.

Evaporation: Though the SciFlow 1000 does have a lid, evaporation can be observed, and for experiments over 7 days, a 10 – 20% larger volume can be added to the source well than is removed from the sink, to combat decreasing volumes in the source.

Sampling: It is possible to sample from the wells during the experiment. 5 – 10μl aliquots can be removed from each well in a row using a multichannel pipette, to simultaneously remove sample from all of the wells.

Running Assays in SciFlow: Cell based assays can be run in the SciFlow 1000. It is important to remember that once the fluidics are engaged they will stay engaged. Here are a few assay suggestions:

- For endpoint assays remove all media from the plate by inversion and flicking the plate over an appropriate waste container.
- Keep volumes of buffer and assay reagents less than 50μl in order to reduce possible flow from well to well.
- Shorter incubation times are preferable, minimizing inter-well flow.

Sample Plate Map:

	Source	2	3	4	5	6	7	8	9	10	11	Sink
A	Fluorescein Tracer		Acellular Fluidic Tracer									
B	Compound 1 (triplicate rows)		Cellular Model (e.g. HepaRG cells @ 40,000 cells/well)									
C												
D												
E	Vehicle control (triplicate)		Cellular Model (e.g. HepaRG cells @ 40,000 cells/well)									
F												
G												
H	Fluorescein Tracer		Acellular Fluidic Tracer									

Example Cell Seeding Densities

Table of Example Cell Seeding Parameters: SciFlow culture well areas are ½ the size of traditional 96-well culture surface areas (0.167cm^2 or 16.7mm^2).

Cell Seeding Examples, 2D monolayers	Number of cells per SciFlow plate	Number of cells per well	How many culture wells	Seed Time	Initial confluence	Adjustment
Primary human hepatocytes with collagen coating	2.0 E6	27,500	72 (3 – 11)	Overnight	Confluent	By viewing
Primary rat hepatocytes with collagen coating	1.0 E6	14000	72 (3 – 11)	Overnight	Confluent	By viewing
Primary mouse hepatocytes with collagen coating	6.5 E5	9000	72 (3 – 11)	Overnight	Confluent	By viewing
Primary duck hepatocytes with collagen coating	6.5 E5	9000	72 (3 – 11)	Overnight	Confluent	By viewing
Primary canine hepatocytes with collagen coating	1.0 E6	14000	72 (3 – 11)	Overnight	Confluent	By viewing
HepG2	2.2 E6	30000	72 (3 – 11)	Overnight	80%	By viewing
HepaRG	2.9 E6	40000	72 (3 – 11)	Overnight	80%	By viewing
HepaRG (no spin)	2.9 E6	40000	72 (3 – 11)	Overnight	80%	By viewing
Cell Line MCF7	7.2 E5	10000	72 (3 – 11)	Overnight	20%	By viewing