Evaluation of Cells Shipped within Assay Plates in a Semi-Solid Shipping Gel – 5 Days in the Mail

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Introduction

Mammalian cells are usually transported frozen in cryovials on dry ice or in liquid nitrogen vapour over long distances by specialist couriers. On arrival, the culture of these cells to a viable population useful for assay purposes takes days to weeks, often with considerable variability. Many vendors provide 'assay ready' cryo-preserved division arrested cells, but these can be difficult to resurrect to reproduce the quality of data seen on the product insert sheet. In addition, the hazardous nature of dry ice and liquid nitrogen, and the associated high shipping cost, severely limit their routine use. In this study, we tested the properties of cells after being preserved and shipped over long distance over 5 days at 10 to 20°C in a semi-solid gel within micro-well plates (WellReady™ by Atelerix). We evaluated the quality of the cells using a number of assay formats including a firefly based luminescence reporter assay, a BRET based kinase cellular assay and a FLIPR calcium flux assay. Our data suggests that cells maintained in WellReady™ during shipping over a 5 days period responded in a similar manner to stimulation as cells that had not been shipped. This data raises the possibility of shipping other cell types such as primary cells and differentiated stem cells in well plates in a manner such that, on arrival, cells could be cultured for 4 to 24hrs then used for bio-assays in a quick and convenient manner.

Methods

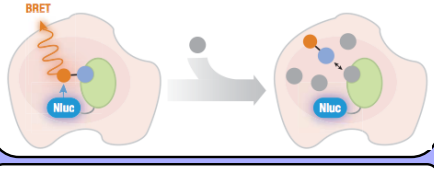
HEK293 cells were cultured and/or plated in DMEM (high glucose – Gibco) + 10% FBS in all experiments into either $T_{75}cm^2$ flasks or appropriate 96 or 384 wells plates (white solid for luminescence and BRET, black transparent for FLIPR). Where necessary, cells were detached with TrypleE (Gibco), counted using an automated counter, and plated using a multi-channel dispenser (Integra).

For WellReady™ gel experiments, media was removed and replaced as described in the table below using an automated 16 channel dispenser or ViaFlo384 (Integra) set to very low aspirate and dispense speed.

Manipulation step	96 well (µl addition/well)	384 well (µl addition/well)
Dispense solution A	100	20
Dispense solution B	100	20
Dispense gelation reagent C	50	20
Incubate RT 10mins	-	-
Dispense gelation reagent D	50	20
Incubate RT 10mins	-	-
Remove reagents D and C	-100	-40
Dispense media	200	40
Incubate RT 5mins	-	-
Remove Media	-200	-40
Dispense solution E	100	20
Adhesive seal applied	-	-

Gel was allowed to cure for 4hrs at RT before shipping. Plates were shipped in boxes containing chill packs designed to generate and maintain an ambient internal box temperature of between 10 and 20°C. Boxes were shipped using a routine courier as there are very few restrictions and reduced cost of shipping ambient temperature reagent.

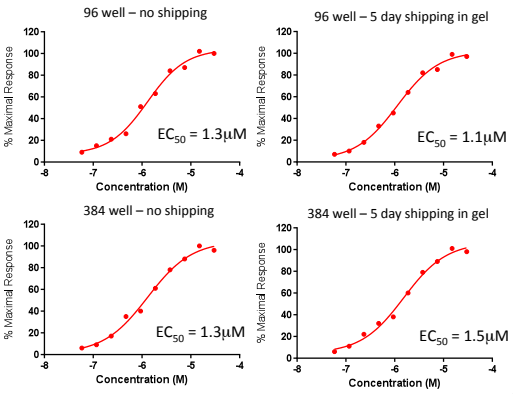
Diagram of Target Engagement Assay – kinase protein (green) is expressed with HiBiT tail. On addition, Nluc binds to tail (blue) plus substrate generate photons. When tracer (blue) binds to kinase, fluorescent label (orange) comes into close proximity and BRET occurs. Compound (grey) binds to kinase and competes off tracer therefore signal decreases.



CyclicAMP-response element based luciferase assay – shipping versus non-shipping

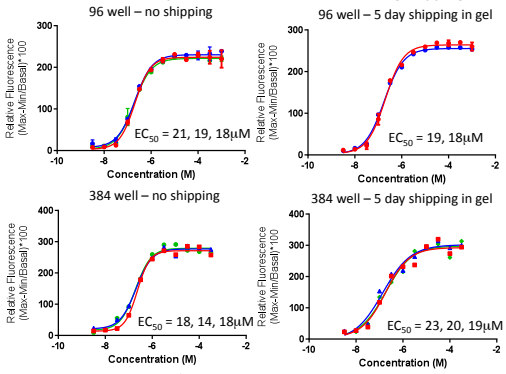
A cyclic AMP-response element (CRE) – firefly luciferase cDNA construct (Promega) was transiently transfected into adherent HEK-293 cells ($T_{75}cm^2$ flask). Following a 24hr incubation period, cells were plated into 96 (30,000/100µl) or 384 (8,000/50µl) well plates. The following day, cells were either treated with a dose-response to forskolin to elevate cyclicAMP levels, detected by an increase in luminescence, or maintained as a adherent monolayer below a semi-solid gel, then boxed and shipped for 5 days (@ 10-20°C). Upon arrival, the gel was removed and the cells incubated in 100µl (96) or 40µl (384) media for 24hrs (37°C/5%CO_2) prior to forskolin treatment and subsequent luciferase measurement. Data indicates that cells remain healthy in plates below the gel for 5 days and, following a 24hr recovery period, are able to respond to forskolin with similar EC_{50} values to non-shipped cells. Consistent data was obtained from either 96 or 384 well plates.

Forskolin dose-response following shipping in 96 and 384 well plates



Calcium flux based FLIPR assay – shipping versus non-shipping

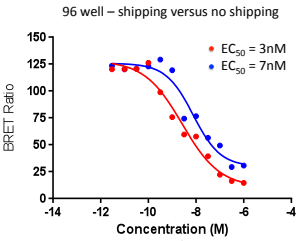
ATP dose-response detected in FLIPR following shipping



HEK-293 cells were plated into 96 (30,000/100µl) or 384 (8,000/50µl) well plates and incubated for 24hrs@37°C. Plates were then divided into two groups, either tested or shipped. For shipping, adherent monolayers of cells were treated with gelling reagents and plates were shipped for 5 days (@ 10-20°C). Following shipping, the gel was removed and plates were incubated for 24hrs @ 37°C/5%CO_2. Media was replaced with assay buffer (HBSS, 20mM HEPES pH7.4) containing Fluo-4 reagent (Molecular Devices) and incubated at 37°C for 60mins. ATP was used to stimulate a calcium flux response via interaction with endogenous purinergic receptors on HEK293 cells. The FLuorescent Imaging Plate Reader (FLIPR - Molecular Devices) was used to dispense ATP at various concentrations from a separate ligand addition plate and simultaneously dispense and detect an increase in fluorescence consistent with calcium mobilisation within the cells.

Data represents mean +/- S.D. of three replicates per concentration on three separate plates (red, blue green). The EC_{50} for ATP on endogenous purinergic receptors in this study is approximately 20µM, consistent with literature values. The is no difference in response between shipped cells (5 days @ 10-20°C) and non-shipped cells either in 96 or 384 well plates.

Kinase based target engagement live cell bind assay – shipping versus non-shipping



HEK293 cells were transiently transfected with a cDNA containing the DDR1 kinase sequence connected to HiBiT, an 11 amino acid sequence of the Nanoluc enzyme (Promega) (24hrs @ 37°C/5%CO_2), in a $T_{75}cm^2$ flask. Cells were plated into two 96 well plate, incubated for a further 24hrs, then a plate was shipped for 5 days (10-20°C). Following shipping, the plate was incubated for a further 24hrs then a target engagement assay was performed (Promega). Briefly, cells are treated with LgBiT, the remaining Nanoluc enzyme plus substrate. LgBiT homologous recombination with HiBiT results in an active Nanoluc enzyme that, in the presence of substrate, generates photons. When a fluorescently tagged tracer binds to the kinase domain it comes into close proximity with Nanoluc and photons active the fluorescent tag, resulting in BRET. Competition with a ligand that binds to DDR1 results in a dose dependent decrease in BRET

Data represents mean +/- S.D. of two replicates per concentration of competing compound Dasatinib (red = non-shipped cells, blue = shipped cells). The EC_{50} for Dasatinib binding to DDR1 between shipped and non-shipped cells was 3nM and 7nM respectively.

Summary and Conclusion

We have demonstrated the use of a semi-solid gel that can be added to and removed from adherent cells and used to maintain the cells in a quiescent state for shipping living cells in either 96 or 384 well plates at 10-20°C. Following de-gelation and incubation for 24hrs in media, the cells recover and can be used for bio-assay purposes. Here, we evaluated HEK293 adherent cultures in two transient transfection assays (cyclicAMP luciferase reporter CRE and target engagement kinase DDR1) together with fluorescent kinetic responses in a FLIPR assay, stimulating endogenous receptors with ATP. In all cases the responses and EC_{50} between shipped and non-shipped cells for the ligands and between 96 and 384 well plates were similar.

The advantages of this system are:

- cells do not need to be cryo-preserved before shipping – saving costs and time/difficulty to resurrect cryo-preserved cells
- cells can be grown at source and shipped in micro-well plates then used at the destination with minimal manipulation
- shipments only require 10-20°C therefore dry ice or wet ice are not required, reducing shipping costs and paperwork
- cells maintain transient expression even after 5 days in gel, suggesting this approach could 'pause' cell before assay

Next steps are to work with iPSC's and cells differentiated from iPSC's to determine if these can be generated in our laboratories and shipped to clients for screening purposes. In addition, human primary cells, either adherent or as a 3-D spheroid, will be evaluated for shipping to clients allowing just in time testing without the need for this work to be completed locally by the client, improving efficiency and quality of well plate cells for compound evaluation.