Evaluation of the PlasmaKnife™ (IonField Systems) for microplate cleaning

Determining the effect of plasma treatment on high value assay plates

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Single-use microtitre plates are a significant cost factor in high throughput screening (HTS) campaigns and for many *in vitro* screening applications. IonField Systems (IFS) are developing the PlasmaKnife™, an automated instrument which uses cold-plasma to clean microtitre plates, achieved through exposure to room-temperature plasma created by a high-voltage dielectric discharge. Treatment is claimed to restore plastic microtitre plates so that they can be reused multiple times, targeting a purchase cost reduction of up to 98% (50 re-uses), as well as cost reductions in total plastic hazardous waste produced.

Proof of principle studies on microplates completed by IFS in early 2014 described application to standard plate types. Data from a preliminary study by AstraZeneca with IFS has now explored a selection of high value plates for more complex assays.

Hence, we present here an evaluation of the effect of the PlasmaKnife™ treatment on electrophysiology, RT-PCR and label-free biosensor plates.

Background to technology

IonPlasma is atmospheric pressure plasma powering the PlasmaKnife system. It is generated by electron streamers that fire off the dielectric barrier when the right frequency and power levels are achieved. The streamers ionize the air and any water vapour. The main reactive products in the ionized gases are ozone, high energy oxygen, singlet oxygen and hydroxyl ions. These species, combined with the dense electron cloud, provides the energy to trigger rapid ionization of organic molecules and the conversion of water and other solvents that may be present in samples from a liquid to a gas state. The rapid ionization and liquid-to-gas conversion cleans microtitre plates for immediate re-use.

Treatment process overview
Step1 – Washing

•Optimized wash removes bulk well contents - reducing concentration of well by 1,000X to 10,000X
•A high pressure, pulsed blade “scrubs” & empties wells.
•Multiple solvents can be used (individually or mixed).
•Mix ratios can be fixed or changed continuously during wash process. Solvents can be heated.
•An air knife is used to remove wash solvents, either retaining liquid layer held by surface tension or drying the plate.

Step 2 – Plasma treatment

•Plasma blown into wells across all surfaces.
•Dry plates can be re-wet immediately before being treated with plasma.
•Labs needing fast cleaning can add module to provide higher O_2 content to plasma generator. O_2 rich air provides 2X-4X more potent plasma.

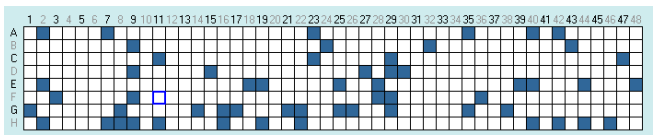
Plate treatment for evaluation at AstraZeneca

•All plates in this evaluation had not been used for screening prior to plasma treatment. Plates were treated with plasma by IFS and sent to AstraZeneca for testing in biological assays.
•Plates washed with water blade using 90% water / 10% alcohol - one surface pass in 30 secs.
•All plates dried with air knife (except EPIC plates - centrifuged at 600 x g) to remove all residual fluid.
•PlasmaKnife™ passed over surface (one pass in 30 secs) – plate repackaged within 2 minutes.

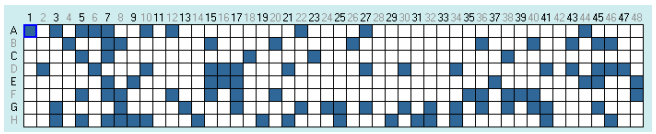
Electrophysiology plates

Single Hole PatchPlates (Molecular Devices) were used in an assay to measuring activity of a potassium channel using both IonWorks Quattro and Barracuda Plus Automated Patch Clamp Systems.

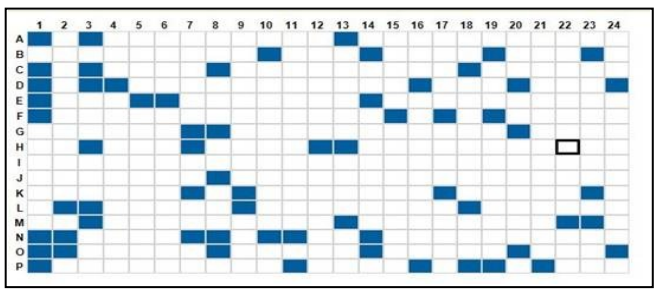
A) Quattro Control Single Hole PatchPlate (No. of usable wells = 327)



B) Quattro PlasmaKnife™ treated Single Hole PatchPlate (No. of usable wells = 289)



C) Barracuda Control Single Hole PatchPlate (No. of usable wells = 320)



D) Barracuda PlasmaKnife™ treated Single Hole PatchPlate (No. of usable wells = 239)

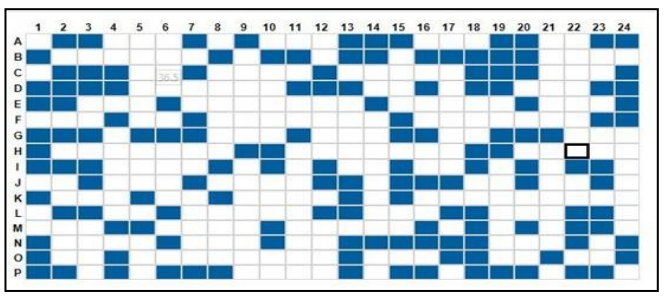
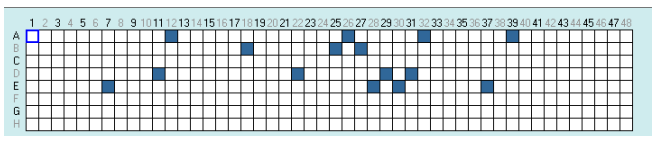


Figure 1. Effect of PlasmaKnife™ treatment on Single Hole PatchPlates in K-channel assay on IonWorks Quattro and Barracuda. CHO cells expressing the K-channel were added to all wells of the PatchPlates. The Patch Clamp System aims to seal a cell to the hole at the bottom of each well. The underside of the PatchPlate is then exposed to Access solution (140mM KCl, 1mM EGTA, 1mM $MgCl_2$, 20mM HEPES 0.1 mg/mL Amphotericin B) in order to obtain a perforated-patch whole-cell recording. The Quattro voltage protocol consisted initially of a 20 sec period clamping the membrane potential to -70 mV prior to a step protocol of a 1 sec step to +40 mV, a 2 second step to -30 mV and finally a 0.5 sec step to -70 mV. A 70 mV holding potential was used throughout the Barracuda experiment due to its ability to continuously clamp the membrane potential, prior to a step protocol of a 1 sec step to +40 mV, a 2 sec step to -30 mV and finally a 0.5 sec step to -70 mV

Population PatchPlates (Molecular Devices) were used in an assay to measure the activity of a voltage-dependent sodium channel using the IonWorks Quattro Automated Patch Clamp System.

A) Control Population PatchPlate (No. of usable wells = 369)



B) PlasmaKnife™ treated Population PatchPlate (No. of usable wells = 315)

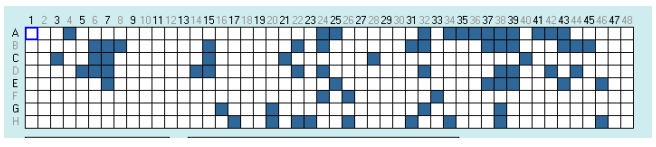


Figure 2. Effect of PlasmaKnife™ treatment on Population PatchPlates in Na-channel assay on IonWorks Quattro. Cells expressing the Na channel were added to all wells of the PatchPlates. The Patch Clamp System aims to seal a cell to the 64-holes at the bottom of each well. The underside of the PatchPlate was exposed to Access solution (100mM CsCl, 1mM EGTA, 3mM $MgCl_2$, 10mM HEPES 0.1 mg/mL Amphotericin B) in order to obtain a perforated-patch whole-cell recording. A “2-pulse” voltage protocol consisting of a 30 ms test pulse to +15mV and 1 sec hold at 0 mV, followed by a repolarisation to -90 mV for 20 ms and a second test pulse to +15 mV, was used. The holding potential was -90 mV, applied for 2 minutes to relieve slow inactivation before the 2-pulse test protocol. A seal resistance filter of 20 MΩ was applied: white wells are usable (> 20 MΩ), blue wells are not.

RT-PCR plates

Roche LightCycler plates were used in an assay to detect the standard housekeeping gene human IPO8.

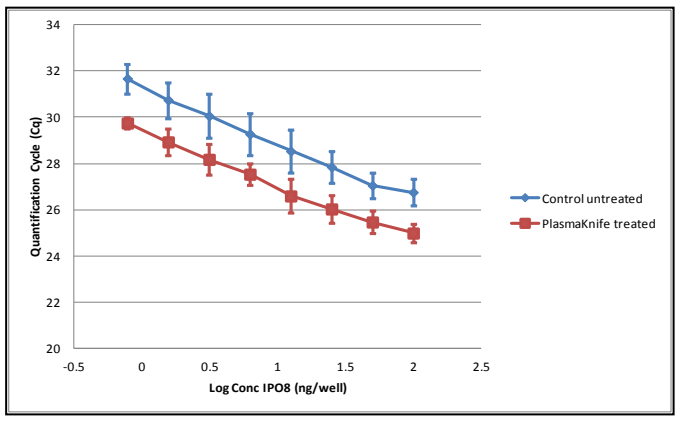


Figure 3. Effect of PlasmaKnife™ treatment on Roche LightCycler 480 plates Using the Transcriptor One-Step RT-PCR Kit (Roche), reactions contained 2 µL 5X RT-PCR reaction buffer, 0.5 µL 20X Assay On Demand primer/probe set for IPO8 (Applied Biosystems) and 0.2 µL Transcriptor Enzyme Mix in a total volume of 8 µL. Human reference RNA (Agilent) was serially diluted in nuclease-free water and 2 µL added per well. The plate was then centrifuged at 600g for 10 seconds. Cycling conditions were as follows: 20 mins at 50ºC and 10 mins at 95ºC, followed by 40 cycles each consisting of 10 sec at 95ºC, 30 sec at 60ºC and 1 sec at 72ºC. Quantification cycle (Cq) data were analyzed using the LightCycler 480 software and plotted against log concentration.

Label-free biosensor plates

Corning Epic un-coated cell plates were coated in house with fibronectin and used in an assay measuring the dynamic mass redistribution response (DMR) of CHO cells overexpressing an orphan GPCR.

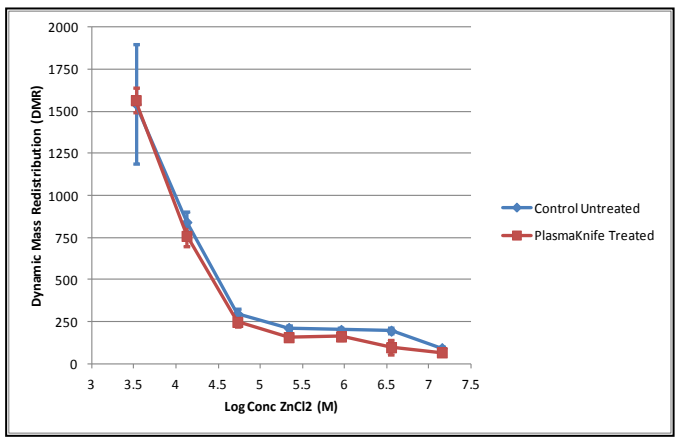


Figure 4. Effect of PlasmaKnife™ treatment on Corning EPIC un-coated cell plates. Plates were coated with 50 µg/mL human fibronectin in PBS and incubated at ambient temperature overnight. The excess liquid was removed centrifugally, and CHO cells over-expressing the orphan GPCR. were added to the well. After overnight incubation at 37°C, 5% CO_2, cell media was exchanged for assay buffer (HBSS containing 2 mM HEPES, 0.6 % (v/v) DMSO) and allowed to equilibrate to ambient temperature for 90 mins. After equilibration within the EPIC for a further 30 mins, plates were read for 30 mins to achieve a baseline measurement, followed by the addition of various concentrations of $ZnCl_2$ in assay buffer. Kinetic DMR data was collected for 90 minutes. Concentration response data shown is calculated from Maximum Peak DMR.

Summary

•The combined wash / PlasmaKnife™ treatment had a deleterious effect on both Single Hole and Population PatchPlates. Potentially the air knife (>100 psi) used to blow off the residual fluid after washing may have deformed the opening in the bottom where the cell is held during testing. We are currently investigating the effect of centrifuging as an alternative approach to remove wash solvent.

• PlasmaKnife™ treated LightCycler plates showed a reduction in sensitivity for detecting the IPO8 housekeeping gene, but the signal:noise was unaffected. The combined wash / PlasmaKnife™ treatment had no negative impact on the Epic uncoated cell plates. The effect of multiple cycles of wash/plasma treatment needs to be investigated to determine the number of times these different plate types could be re-used.

•This preliminary evaluation has highlighted other factors which could be investigated, such as recoating plates post plasma treatment; these may allow application of the PlasmaKnife™ for these, and other microtitre plate types, going forward.

Update 2016 - the air knife was removed from the PlasmaKnife™ beta and release product

IonField™ SYSTEMS AstraZeneca