BD PureCoat™ surfaces: Development of two novel culture surfaces that enhance cell performance in cell-based assays

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Application Note

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Introduction

Cell-based assays are commonly used in drug discovery as well as in basic and clinical research activities. They provide predictive model systems that allow researchers to observe physiological responses of cells to pharmacological agents and environmental conditions. High-throughput cellular screening assays enable rapid analysis of many compounds for their effects on specific targets. The robustness of cellular screening depends on consistency of cell performance which in turn is directly related to cell attachment. BD Biosciences proudly introduces the next generation of advanced cell culture surfaces—BD PureCoat™ cultureware. Encompassing two new chemically defined, animal-free cell culture surfaces, BD PureCoat cultureware is designed to improve cell performance. Produced by a proprietary thin-film coating technology, BD PureCoat cultureware is available in two different surface chemistries—BD PureCoat amine, a positively charged surface and BD PureCoat carboxyl, a negatively charged surface. BD PureCoat surface treatments support better cell attachment than standard tissue culture (TC) surfaces when using transformed, transfected, division arrested (DA), and fastidious cell lines under less than optimal culture and assay conditions such as low or no serum. These surfaces also maintain integrity of cell monolayers during assay liquid handling procedures and, therefore, offer superior consistency for use in cell-based assays. More importantly, cells grown on BD PureCoat surfaces remain functional and continue to perform well in HTS-based assays such as G-Protein Coupled Receptor (GPCR) screening using FLIPR®, Cyclic Adenosine Monophosphate (cAMP), and proteasome-inhibition studies. The surface treatment does not interfere with commonly used cell labeling dyes. BD PureCoat amine and carboxyl surfaces will enable researchers to better optimize culture conditions, which will enhance the performance of cell-based assays.

Materials and Methods

DA cell lines

Cryopreserved hERG-T-REx™ 293 DA cells purchased from Invitrogen (cat. no. K1383) were thawed in a 37°C water bath for 1-2 minutes and transferred into 10 mls of growth medium (DMEM with Glutamax, 0.1 mM non-essential amino acids, penicillin/streptomycin, 25 mM Hepes, and 10% dialyzed serum). Cells were centrifuged at 200 g for 5 minutes and pellet was resuspended in fresh growth medium. An aliquot was removed to obtain a cell count. Cells were seeded at 20,000 or 10,000 cells/well onto 96- or 384-well plates, respectively, and incubated at 37°C in a humidified 5% CO_2 atmosphere. The next day, plates were visualized under a microscope and images captured.

A tetrazolium-based assay was utilized to quantify cell attachment. Briefly, exhausted media was gently removed, replaced with media containing MTS reagent (Promega), and incubated at 37°C for 2 hours. MTS is a tetrazolium compound that is reduced by metabolically active living cells into a soluble product, formazan, that gives a purple hue. The absorbance of formazan at 490 nm was then read on a Tecan® Safire2™ microplate reader. A row of wells containing media + MTS served as an experimental blank.

EcoPack2-293 cell lines

EcoPack™2-293 cell line originally obtained from Clontech was grown in DMEM (4.5 mg/ml glucose) containing 4 mM L-Glutamine, 1 mM sodium pyruvate, and 10% FBS. For this study, cells were trypsinized (0.05% Trypsin-EDTA), centrifuged, resuspended in serum-free growth medium, and seeded at 10,000 cells/well onto 384-well plates. After an overnight incubation at 37°C in a humidified 5% CO_2 atmosphere, plates were visually examined, washed two times with 1X Hanks Buffered Salt Solution (HBSS) containing 10 mM HEPES using an automated microplate washer (Skatron EMBLA) and stained with 2.5 µM calcein AM fluorescent dye. In other experiments, cells were seeded at 2,250 cells/well onto 1536-well plates, stained with calcein AM by Multidrop® Combi (Thermo Scientific) instrument and then washed using AquaMax® (Molecular Devices), an automated liquid handling system. The plates were then read after a 1-hour incubation with dye at room temperature on a PerkinElmer EnVision® microplate reader. Z' values for the assay were quantified.

GPCR assay using calcium mobilization

GPCR assays using EcoPack2-293 cells were performed using a Calcium-4 kit purchased from Molecular Devices (cat. no. R-8141). EcoPack2-293 cells cultured in growth medium [DMEM (4.5 mg/ml glucose) containing 4 mM L-Glutamine, 1 mM sodium pyruvate and 10% FBS] were trypsinized (0.05% Trypsin-EDTA), centrifuged, and seeded on 384-well plates at 10,000 cells/well in 25 µl of serum-free growth medium. After an overnight incubation at 37°C in a humidified 5% CO_2 atmosphere, cells were labeled with 25 µl of dye loading buffer (Calcium-4 kit) containing 2.5 mM probenecid for 1 hour followed by addition of 12.5 µl of buffer or 5X drugs (carbachol and ATP made from stock solutions in water) for receptor activation. These GPCR activation assays were carried out on a FlexStation® 3 multi-mode benchtop reader (courtesy of Molecular Devices).

cAMP assay

cAMP levels in HEK-293 cells were measured using a Catchpoint® fluorescent assay kit (Molecular Devices; cat. no. R8088). This cell line originally obtained from ATCC was cultured in DMEM (4.5 mg/ml glucose) growth medium containing 4 mM L-Glutamine, 1 mM sodium pyruvate, and 10% FBS. To initiate the study, cells were trypsinized (0.25% Trypsin-EDTA) and seeded onto 96-well plates at 100,000 cells/well in serum-free growth medium. The next day, cells were washed with Krebs Ringer Buffer (Sigma; cat. no. K4002) and incubated in 100 µl of Stimulation Buffer containing 0.75 mM IBMX, a phosphodiesterase inhibitor (Sigma; cat. no. I7018) for 10 minutes at room temperature according to manufacturer's instructions. Each well was then treated with 50 µl of 3X Isoproterenol (Sigma; cat. no. I5627) or PBS for 15 minutes at 37°C. The final concentration of Isoproterenol in each well was 20 µM. Cell lysis was achieved by addition of lysis reagent (50 µl/well) followed by mechanical agitation for an additional 10 minutes. A standard curve was constructed using calibrators provided in the kit and cAMP levels in lysates were measured according to manufacturer's recommended protocol.

Proteasome Inhibition Assays by Fluorometry

Living Colors™ HEK-ZsGreen Proteasome sensor cell line was purchased from Clontech (cat. no. 631535). It is a clonal human embryonic kidney cell line that stably expresses ZsProSensor-1 and is utilized to detect changes in proteasome activity, which is reflected as alterations in green fluorescent protein levels. Cells were maintained on BD BioCoat™ Collagen I 75 cm^2 flasks (cat. no. 356485) in DMEM growth medium containing 4.5 mg/ml glucose, 4 mM L-Glutamine, 1 mM sodium pyruvate, penicillin/streptomycin, 0.2 mg/ml G418, and 10% FBS. To initiate this study, cells were trypsinized (0.25% Trypsin-EDTA) and seeded onto 96-well plates at 30,000 cells/well in growth medium. After an overnight incubation at 37°C in a humidified 5% CO_2 atmosphere, each well received 5 µl of 20X Ac-Leu-Leu-Nle (ALLN), a calpain inhibitor that inhibits proteasome activity by preventing the degradation of ZsProSensor-1 protein. Final concentrations of ALLN in wells ranged from 0.1-10 µM whereas control (background) wells received an equal volume of vehicle (DMSO). Twenty hours post treatment, plate fluorescence was read at 483 nm on a Tecan Safire2 microplate reader. A row of untreated cells served as the blank for the experiment and signal-to-noise (STN) ratios were calculated for the assay.

Results and Discussions

Cell-based assays are a dynamic tool for drug discovery platforms and reproducibility of data is vital in cellular screening assays. To minimize variation among replicate samples, there is need to culture cells in optimized and defined conditions. Here, we report new enhanced cell culture surfaces, BD PureCoat surfaces, that provide improved, consistent, and uniform cell attachment for transfected and transformed cell lines subjected to standard or rigorous assay conditions.

DA cell lines are assay-ready reagents and facilitate high-throughput cellular screening in functional drug discovery assays. We have successfully demonstrated that BD PureCoat amine significantly increases cell attachment of hERG-T-REx 293, a DA cell line, over TC-treated and Competitor C surfaces. Results from a representative experiment indicate that ≥ 40% more cells attached on BD PureCoat amine than TC-treated or Competitor C plates (Figure 1A).

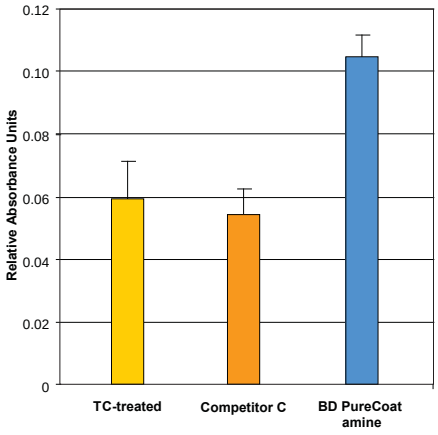

Figure 1A.

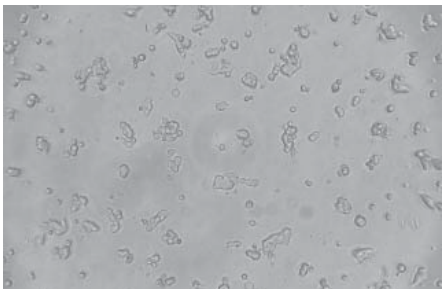

Figure 1B-1. TC-treated.

Figure 1B-2. BD PureCoat amine

Figure 1. Better attachment and freeze-thaw recovery of hERG-T-REx 293 DA cells on BD PureCoat amine.

Cryopreserved hERG-T-REx 293 DA cells were thawed in a 37°C water bath and immediately seeded onto 96-well black/clear TC-treated, Competitor C, or BD PureCoat amine plates in growth media at 20,000 cells/well. After an overnight incubation, exhausted media was removed and replaced with growth medium containing MTS reagent (Promega) for 2 hours. Relative absorbance units (Signal-Blank values; mean ± SD, n=10 wells/surface) from a representative experiment show that values are highest on BD PureCoat amine compared to those on TC-treated or Competitor C plates (Figure 1A). Cells post-thaw were also seeded onto 384-well black/clear plates at 10,000 cells/well in growth medium. Images of random fields viewed by light microscopy were captured at 100X magnification. Note: even attachment of cells on BD PureCoat amine, whereas those on TC-treated plates are fewer and appear clumpy (Figure 1B1-2).

Furthermore, images of cells grown on BD PureCoat amine show homogenous attachment and appear more confluent and less clumpy than TC-treated plates (Figure 1B). Cell clumping is often associated with increased variation and decreased assay reliability. The stringency of cell attachment was further tested by introducing wash steps. Towards this end, EcoPack2-293 cells cultured in serum-free medium (384- and 1536-well formats) were washed two times with a buffered salt solution using an automated liquid handling system. This allowed us to test the robustness of cell attachment on TC-treated vs. BD PureCoat surfaces. Pre- and post-wash images indicate that cells are washed off from either the TC-treated or the Competitor C surfaces, whereas the cell monolayer remained intact on BD PureCoat amine (Figure 2A).

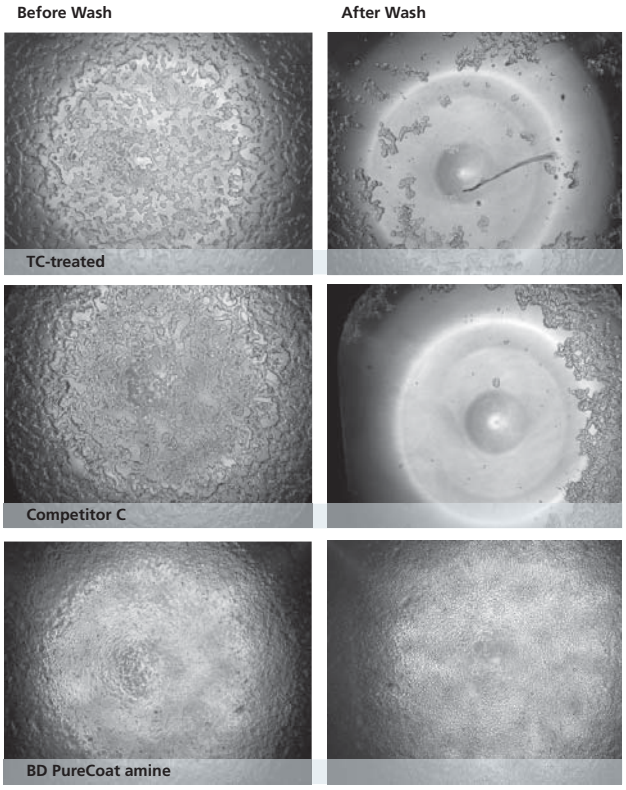

Figure 2A

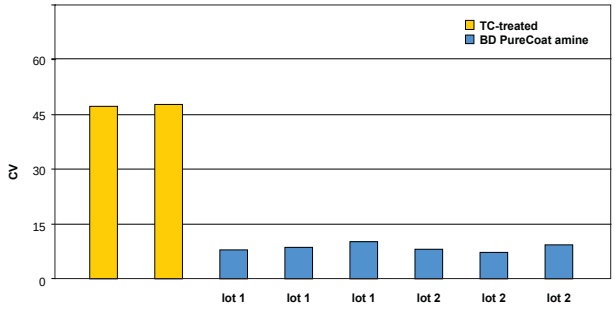

Figure 2B-1. 1536-well.

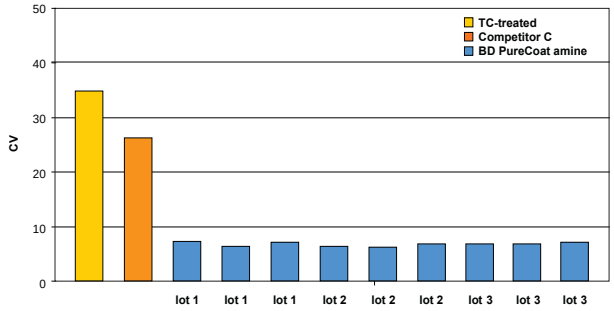

Figure 2B-2. 384-well

Figure 2. Enhanced attachment of EcoPack2-293 cells, a viral packaging cell line on BD PureCoat Amine.

Cells were seeded onto 384- or 1536-well black/clear BD PureCoat amine, TC-treated, or Competitor C plates at 10,000 cells/well and 2,250 cells/well, respectively, and grown under serum-free conditions for 20-24 hours. The cells were then washed (on a Skatron EMBLA washer) two times with HBSS containing 10 mM Hepes, loaded with calcein AM for 1 hour and read on a PerkinElmer EnVision plate reader. As shown in Figure 2A, pre- and post-wash images indicate that cells remain attached on BD PureCoat amine surfaces and are washed away on other surfaces tested (384-well format). Intra-plate CVs of multiple lots of BD PureCoat amine were < 10% for 384- and 1536-well plate formats, whereas CV's for TC-treated or Competitor C plates were much greater (Figure 2B) indicating superior reproducibility in cell-based assays on BD PureCoat amine surfaces.

Cell attachment was further quantified by calcein AM fluorescent dye stain. Relative Fluorescent Units and Coefficient of Variance (CVs) of the assays were compared in multiple lots of BD PureCoat amine plates vs. TC-treated or Competitor C surfaces. Among BD PureCoat amine plates, intra-plate CVs were < 10% for both 384- and 1536-well formats, whereas CVs for TC-treated and Competitor C plates ranged from > 25-45% (Figure 2B). Z' values, a predictor of assay reliability, was calculated for 384- and 1536-well formats and determined to be > 0.72.

Cells cultured on BD PureCoat surfaces remain functional and can be utilized for GPCR assay development. We assessed the performance of BD PureCoat amine surface in a GPCR assay by detection of calcium mobilization using EcoPack2-293 cells in response to carbachol and ATP, a muscarinic and P2X7 receptor agonists, respectively. Agonist-induced increase in cytosolic calcium in cells grown on BD PureCoat amine are similar to those grown on TC-treated plates (Figure 3, A-B).

Moreover, the potency (EC_{50}) of tested drugs on BD PureCoat amine is comparable to cells grown either on BD PureCoat amine or TC-treated surfaces. These results suggest that BD PureCoat amine surfaces can be easily incorporated into existing GPCR platforms.

Functional assays for cAMP, another second messenger molecule, were also evaluated in cells cultured on BD PureCoat surfaces. We chose HEK-293 cells, a human cell line commonly used in drug discovery research because endogenous levels of adenylate cyclase activity are low. Specifically, cAMP responses in HEK-293 cells stimulated with Isoproterenol, a β-adrenoreceptor agonist, cultured on BD PureCoat surfaces in serum-free medium was quantified using a Catchpoint fluorescent assay kit. Results demonstrate that cells cultured on BD PureCoat amine and carboxyl surfaces remain functional and exhibit agonist-induced cAMP responses that are greater than that observed on TC-treated surfaces (Figure 4). Optimal cell attachment to culture surfaces is a

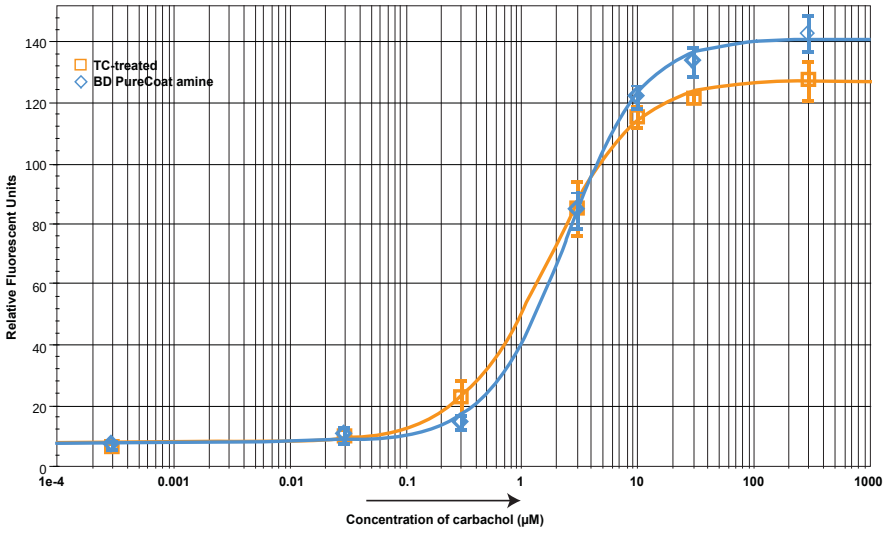

Figure 3A.

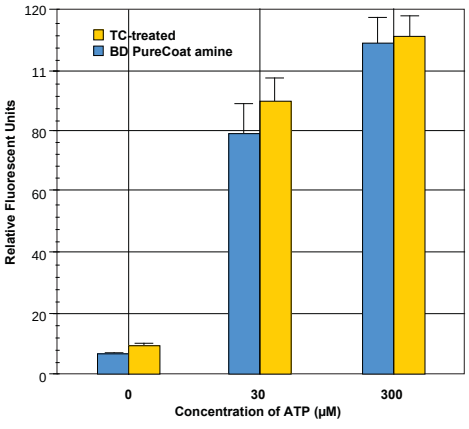

Figure 3B.

Figure 3A-B. Cells on BD PureCoat surfaces remain functional and can be coupled for use in GPCR-based assays.

Cells on BD PureCoat surfaces remain functional and can be coupled for use in GPCR-based assays. EcoPack2-293 cells were seeded onto 384-well black/clear BD PureCoat amine or TC-treated plates at 10,000 cells/well. Twenty-four hours later, cells were incubated with 25 µl of dye loading buffer (Calcium-4 kit) containing 2.5 mM probenecid for 1 hour at 37°C in a humidified 5% CO2 atmosphere. Cells were then treated with varying concentrations of carbachol or ATP and agonist-induced increase in cytosolic calcium was recorded 30 seconds after compound addition on a FlexStation 3. Carbachol induced a sigmoidal dose-dependent response in cells grown on BD PureCoat amine as well as TC-treated as shown in Figure 3A similar response was also seen in cells treated with ATP as shown in Figure 3B (n=4 wells/treatment).

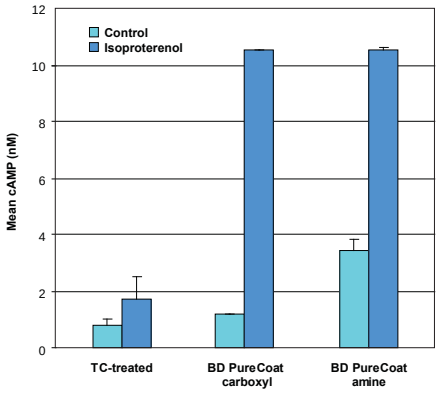

Figure 4. Utilization of BD PureCoat surfaces for functional second messenger cAMP assays.

HEK-293 cells were seeded onto 96-well black/clear TC-treated and BD PureCoat surfaces at 100,000 cells/well in serum-free media. The next day cells were washed, treated with isoproterenol for 15 minutes, lysed, and assayed for cAMP according to manufacturer's instructions (Invitrogen). Agonist-induced cAMP responses were greater in cells grown on BD PureCoat surfaces than TC-treated plates (n= 6/treatment/surface). Z' values were greater on BD PureCoat surfaces (>0.8) than TC-treated surfaces (< 0.1) and is indicative of a robust assay.

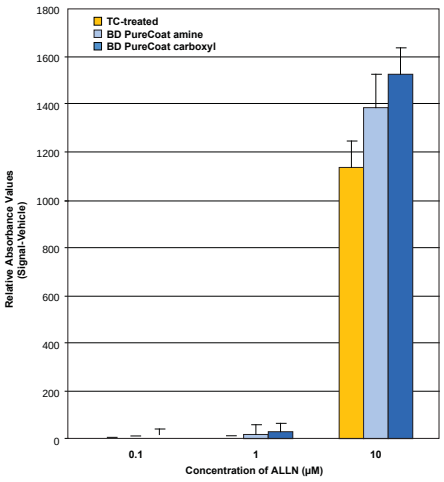

Figure 5. Cells on BD PureCoat surfaces remain functional and can be used for proteasome-inhibition assays.

HEK-293 ZsGreen Proteasome Sensor Cells were seeded onto 96-well black/clear TC-treated and BD PureCoat surfaces at 30,000 cells/well in growth medium supplemented with 10% serum, 0.2 mg/ml G418, and incubated at 37˚C in a humidified 5% CO_2 atmosphere for 20-22 hours. The next day, cells were treated with varying concentrations of ALLN, a proteasome inhibitor or vehicle (DMSO; n=10 wells/treatment). Each well received 5 µl of the test substance yielding the desired concentration. The plates were then read on a Tecan microplate reader at 483 nm. Results from a representative experiment indicate that cells plated on BD PureCoat surfaces remain functional and respond to ALLN.

key factor for determining consistent and reliable cAMP levels in this assay. The single wash step in this assay dislodged cells plated on TC surfaces, but cell monolayers remained intact on BD PureCoat amine and carboxyl surfaces. Subsequently, cAMP response to Isoproterenol was significantly greater in cells cultured on BD PureCoat surfaces than TC-treated plates. This response to Isoproterenol in HEK-293 cells indicates that the cells are robustly attached and remain functional on BD PureCoat surfaces, even under less than optimal culture conditions with no serum. The Z' values were < 0.1 and > 0.8 on TC-treated and BD PureCoat surfaces, respectively. The latter values are indicative of a reproducible assay and further reinforces the superior consistency provided by BD PureCoat surfaces in cell-based assays.

These surfaces have also been tested in proteasome-based assays using an HEK-293 ZsGreen Proteasome Sensor Cell Line (Clontech). Proteasomes are involved in the protein degradation machinery, and are relatively new targets for cancer therapy. In the HEK-293 ZsGreen proteasome sensor cell line, basal fluorescent levels are low because ZsGreen protein is degraded by proteasomes. However, in the presence of proteasome inhibitors such as ALLN, the fluorescent protein is not degraded and accumulates in the cell. Here, we demonstrate that cells cultured on BD PureCoat surfaces respond to treatment with ALLN. Inhibition of proteasome activity was quantitatively monitored by fluorometry and reported as an increase in fluorescence values in ALLN-treated vs. untreated cells (Figure 5). This response was comparable in cells plated on BD PureCoat surfaces and TC-treated plates.

The Z' values ranged from 0.57 and 0.7 for BD PureCoat amine and carboxyl plates, respectively. Although background values were slightly higher on BD PureCoat surfaces, STN ratios of cells treated with 10 µM ALLN were > 20 indicating a robust assay.

Conclusions

- BD PureCoat surfaces provide robust and better cell attachment of transfected and transformed cell lines than standard TC-treated surfaces.
- BD PureCoat surface treatment supports attachment and growth of fastidious cell lines under less than optimal culture and assay conditions such as low or no serum.
- BD PureCoat surfaces provide better post-thaw recovery of cryopreserved DA cell lines.
- BD PureCoat surfaces maintain integrity of cell monolayers during assay liquid handling procedures and, therefore, offer superior consistency for use in cell-based assays.
- Cells grown on BD PureCoat surfaces continue to function in HTS-based drug discovery assays such as proteasome inhibition, GPCR activation, and cAMP-based assays.

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