# Increased cell yields of LnCAP, BHK-21, and MRC-5 cells grown on BD PureCoat surfaces

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Application Note

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## Introduction

Cell culture plays a dynamic role in the discovery and development of new drugs. Increased cell yields are therefore beneficial for a varied number of reasons and modified surfaces have been reported to improve cell adhesion and proliferation. BD PureCoat™ surfaces increase cell recovery post freeze-thaw and enhance cell attachment and growth. BD PureCoat surfaces have also been shown to provide a good substratum for growth of cells in reduced-serum containing growth medium. This is particularly attractive as serum components have been shown to interfere in cell-based assays and can result in decreased experimental consistency. Therefore, reduction of serum has become an important requirement for many applications including transfections, protein production and drug discovery. Furthermore, BD PureCoat surfaces provide a uniform and functionalized surface for growth of cells and consistency of surface treatment. Here, we demonstrate increased cell yields of LnCAP, BHK-21, and MRC-5 cells grown on BD PureCoat surfaces when compared to standard tissue culture vessels.

## Methods and Results

To demonstrate the performance of BD PureCoat surfaces, we studied cell growth in using three different cell lines (LnCAP, MRC-5, and BHK-21) grown on these surfaces in complete growth medium as well as reduced serum containing growth medium. LnCAP cells, a prostate cancer cell line originally obtained from ATCC, were expanded and cryopreserved in liquid nitrogen at an early passage in 90% fetal bovine serum (FBS) and 10% DMSO. To initiate this experiment, cryopreserved LnCAP cells were thawed in a 37°C waterbath and immediately seeded at 8,800 cells/cm$^2$ onto 75 cm$^2$ tissue culture-treated (TC) and BD PureCoat carboxyl flasks and cultured in growth medium (RPMI-1640+ L-glutamine+10% FBS) at 37°C in 5% $CO_2$. After 24 hours, spent medium was aspirated to remove trace amounts of the cryopreservant, DMSO, and replaced with 16 ml of fresh growth medium. Cells were allowed to grow for an additional 5 days with a medium change at 4 days post-plating. On day 6, exhausted medium in the flasks the was aspirated, washed with 1X phosphate-buffered saline and harvested using 0.05% Trypsin-EDTA. Cell counts were enumerated on a hemocytometer and viability was assessed by trypan blue exclusion.

**Figure 1A.**

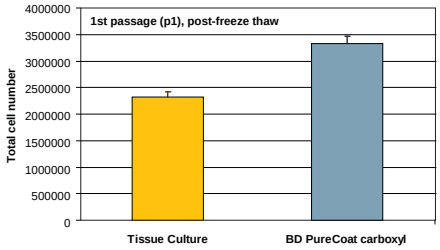


**Figure 1B.**

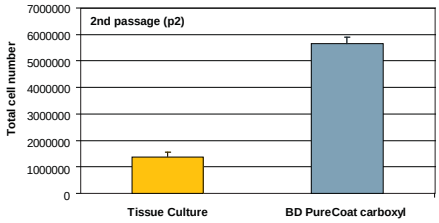


**Figure 1A & B.** Better freeze-thaw recovery of LnCAP, a prostate cancer cell line, on BD PureCoat carboxyl. Early passage cryopreserved LnCAP cells were thawed in a 37°C waterbath and immediately seeded onto TC and BD PureCoat carboxyl 75 $cm^2$ flasks at 665,000 cells/flask in growth medium (RPMI-1640, L-glutamine, 10% fetal bovine serum). After an overnight incubation, growth medium was refreshed and cells allowed to grow. On the $4^{th}$ day, exhausted medium was aspirated and replenished with 16 ml of fresh growth medium. Cells were allowed to grow for an additional 2 day period and later subjected to trypsinization (0.05% Trypsin-EDTA; 2 ml/flask) for 5 minutes at room temperature. An equal volume of growth media was added to neutralize trypsin and the cells were centrifuged and subjected to cell counting (P1). Results from a representative experiment show ≥ 140% increase in LnCAP cell growth post-thaw attached on BD PureCoat carboxyl vs. TC (n=3 flasks/surface). A 1:10 cell dilution of each flask type was passaged and allowed to grow for an additional 6 days in growth medium. Then, cells were trypsinized and quantified using a hemocytometer. This difference was further enhanced upon passaging (P2) resulting in ≥ 312% on BD PureCoat carboxyl flasks vs. TC.

As shown in Figure 1A, there is a marked increase in cell growth on the BD PureCoat carboxyl surface over standard tissue culture-treated (TC) flasks post-freeze thaw. This increase was found to be 43% vs. TC based on an average of three flasks per experiment. Next, a 1:10 dilution of harvested cells were re-seeded onto new 75 $cm^2$ flasks of BD PureCoat carboxyl or TC surface and cultured in growth medium for an additional 6 days. As shown in Figure 1B, continued robust growth of LnCAP cells on BD PureCoat carboxyl flasks was observed. The cell yield from passaged cells grown on BD PureCoat carboxyl surface was 312% greater than standard TC flasks. Here, we demonstrate better freeze-thaw recovery and enhanced growth of LnCAP cells on the BD PureCoat carboxyl surface.

We also evaluated growth of MRC-5 cells, a lung-derived primary cell line cultured on BD PureCoat surfaces. To initiate this experiment, MRC-5 cells grown on standard TC flasks were trypsinized and seeded at 5,000 cells/$cm^2$ onto 75 $cm^2$ TC-treated and BD PureCoat carboxyl surface flasks (n=3 flasks/surface) and cultured in reduced-serum containing growth medium (MEM containing L-glutamine, sodium pyruvate and 5% FBS). After 72 hours, cell confluency measurements on BD PureCoat carboxyl surface and TC flasks were read on an Incucyte™ device (Essen Instruments). As shown in Figures 2A -1 and 2A-2, images captured on the Incucyte show a greater number of cells on BD PureCoat carboxyl vs. TC surface. This result is further reflected in the percent (%) confluency of MRC-5 cell growth on the BD PureCoat carboxyl surface in comparison to growth on TC flasks (70% on carboxyl vs. 50% on TC , Figure 2B). Cells were trypsinized (0.25% Trypsin-EDTA) and counted using the *Vi-Cell*™ XR instrument (Beckman Coulter). Cell yield post-thaw was greater on the BD PureCoat carboxyl surface when compared to standard TC flasks (Figure 2C).

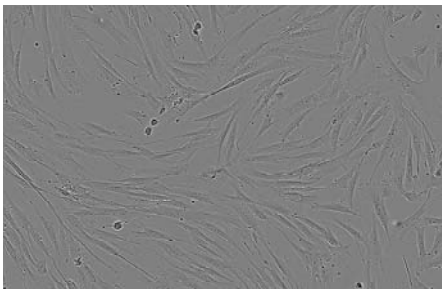

**Figure 2A-1.** BD PureCoat carboxyl

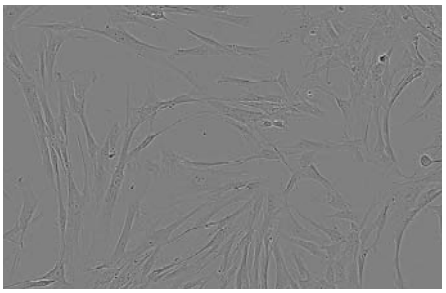

**Figure 2A-2.** Tissue Culture

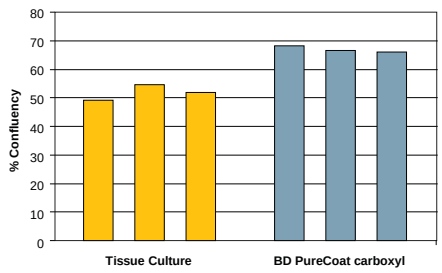


**Figure 2B.**

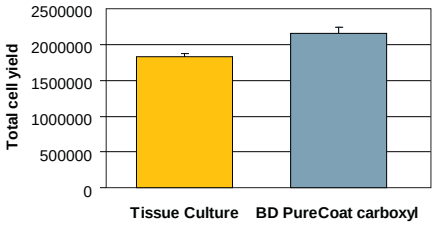


**Figure 2C.**

**Figure 2A-C.** Enhanced cell growth of MRC-5, a lung-derived fibroblast cell line, in reduced serum on BD PureCoat carboxyl. MRC-5 cells were cultured on 75 $cm^2$ TC-treated or BD PureCoat carboxyl flasks seeded at 5,000 cells/$cm^2$ in 15 ml of growth medium (MEM supplemented with sodium pyruvate and 5% FBS). 72 hours later, flasks were subjected to live-cell imaging using the Incucyte™ (Essen Instruments). This assay is a test of confluency levels within flasks and is indicative of area covered by cells (Figure 2A-1 and 2A-2). MRC-5 cells grown on the BD PureCoat carboxyl surface are more confluent than TC flasks (~16% vs. TC; n=3 flasks/surface) as shown in Figure 2B. Then, cells were detached by trypsin (3 ml of 0.25% Trypsin-EDTA/flask) and enumerated on an automated cell analyzing device. Data from a representative experiment is shown in Figure 2C and indicates a mean increase of ≥ 14% in cell yield on BD PureCoat carboxyl surface vs. TC flasks in reduced serum-containing growth medium (n=3 flasks/surface).

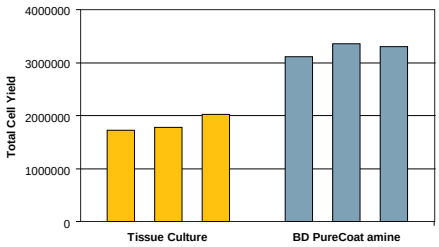


**Figure 3.** Enhanced cell growth of Baby Hamster Kidney (BHK-21) cells in reduced serum on BD PureCoat amine. BHK-21 cells were seeded on TC or BD PureCoat amine 75 $cm^2$ flasks seeded with 1.125 x $10^6$ cells in 15 ml of growth medium (GMEM supplemented with 5% tryptose phosphate broth and 1% FBS). 72 hours later, cells were subjected to trypsinization (3 ml of 0.25% Trypsin-EDTA/flask) followed by neutralization with growth medium, now containing 10% FBS. Cells were centrifuged, the pellet resuspended in growth medium and cell counts enumerated using a hemocytometer. Data from a representative experiment indicate ≥ 76% increase in cell growth was observed on BD PureCoat amine vs. TC-treated flasks in reduced serum-containing growth medium.

Next, we demonstrate that the BD PureCoat amine surface supports growth of BHK-21, a hamster kidney cell line (Sigma Aldrich) in reduced serum containing culture medium. To initiate this study, BHK-21 cells were grown on 175 $cm^2$ tissue culture-treated (TC) flasks in GMEM growth medium containing 5% tryptose-phosphate broth and 10% FBS. On the day of the experiment, cells were trypsinized (0.25% Trypsin-EDTA) and seeded at ~1.1 million cells onto 75 $cm^2$ TC-treated and BD PureCoat amine surface flasks (n=3 flasks/surface) and grown in growth medium, now containing 1% FBS. After 72 hours, spent medium was aspirated and 3 ml of 0.25% trypsin-EDTA was added to each flask and incubated at 37°C for 5-7 minutes. Cell counts and viability were enumerated using a hemocytometer and trypan blue, respectively. Cell yields were greater on BD PureCoat amine than TC-treated flasks. On average, we observed a 76% increase in cell growth on BD PureCoat amine vs. standard TC-treated flasks in reduced serum containing growth medium, as shown in Figure 3.

## Conclusions

The BD PureCoat carboxyl surface supports better freeze-thaw recovery and enhanced growth of LnCAP cells when compared to standard TC-treated surfaces, as well as enhanced growth of MRC-5 cells in reduced serum medium. The BD PureCoat amine surface supports enhanced growth of BHK-21 cells in growth medium containing 1% FBS.


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