# BD PureCoat ™ Amine and Carboxyl Cell Culture Surfaces

***Guidelines for Use***

**BD Biosciences** – Discovery Labware
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## INTENDED USE

BD PureCoat™ amine, a positively charged surface, and BD PureCoat carboxyl, a negatively charged surface, are enhanced tissue culture surfaces. Manufactured using a proprietary thin-film coating technology, these surfaces provide improved attachment, increased proliferation, enhanced recovery from freeze-thaw for a broad range of primary, transfected and fastidious cells that exhibit poor attachment properties on standard tissue culture (TC) surfaces in serum-free/serum-reduced conditions.

## STORAGE AND STABILITY

Store at room temperature (15-30°C).

Stable for at least 12 months from date of manufacture, please refer to product label for expiration date.

## PRODUCT SPECIFICATIONS

BD PureCoat amine and carboxyl surfaces are manufactured using a validated process.

Products are sterilized to SAL $10^{-6}$ by gamma irradiation, and are non-cytotoxic and non-pyrogenic.

Products are quality control tested using an appropriate cell line.

Cultureware material is polystyrene suitable for cell culture.

96-well and 384-well microplates meet ANSI/SBS standards (1-2004, 2-2004, 3-2004, 4-2004) and 1536-well microplates meet ANSI/SBS standards (1-2004, 3-2004, 4-2004).

Please visit http://regdocs.bd.com/regdocs/searchCOA.do for a lot specific Certificate of Analysis.

## GENERAL INFORMATION

### Seeding of cells

All procedures should be performed under aseptic conditions. The optimal seeding density for your cell type on BD PureCoat amine and carboxyl surfaces will need to be optimized in order to achieve the best results. Seeding density is dependent on cell type, media conditions (i.e. serum concentration), and incubation time and detection sensitivity. To determine the best surface and seeding densities for your application, please refer to Table 1.

Cells on BD PureCoat Amine and Carboxyl surfaces do not require special adaptation steps when transitioning from tissue culture (TC) surfaces.

**Table 1: Recommended Surfaces and Seeding Densities for Select Cell Types**

| Cell Type | BD PureCoat Surface Recommendation | Seeding Density (cells/cm$^2$) | Serum Condition | Assay |
|---|---|---|---|---|
| *Primary Neuronal Cells* | | | | |
| Rat Cerebellar Granule | Amine | 315,000-450,000 | 10% FBS | 24-48 hour attachment and differentiation immediately post primary isolation |
| Rat cortical neurons | Amine | As recommended by vendor | Defined medium with growth factors | Freeze-thaw recovery followed by attachment and differentiation for 5 days |
| *Primary Cells* | | | | |
| Rat Primary Brain Astrocytes | Amine | 10,000 | Medium contains 3% serum and growth factors provided by vendor | Freeze-thaw recovery followed by 6 day proliferation in defined medium |
| *Transfected Cells* | | | | |
| EcoPack™2-293 | Amine | 91,000 | Serum-free | One day attachment with a wash step, a GPCR assay has also been performed after one day attachment at this seeding density |
| Living Colors™ HEK-sGreen Proteasome Sensor | Amine or Carboxyl | 93,750 | 10% FBS | One-day attachment, then stimulate with proteasome inhibitor (ALLN) for 20h |
| hERG-T-REx™ 293 Division Arrested Cells | Amine | 62,500 | 10% FBS | Freeze-thaw recovery, then one-day attachment |
| *Cell Lines* | | | | |
| BHK-21 | Amine | 10,000-20,000 | 1% FBS | Three day proliferation |
| HEK-293 | Amine | 312,500 | Serum-free | One day attachment followed by stimulation with cAMP agonists |
| HepG2 | Amine | 25,000 | 10% FBS | One-day attachment, then transfection using Lipofectamine 2000 |
| HepG2 | Carboxyl | 10,000-20,000 | 5-10% FBS | Four day proliferation |
| HT-1080 | Carboxyl | 25,000 | Serum-free | Overnight attachment |
| HT-1080 | Carboxyl | 25,000 | 10% FBS | Overnight attachment followed by transfection with ZsGreen plasmid DNA using Lipofectamine 2000 |
| LnCAP | Carboxyl | 50,000 | 10% FBS | Freeze-thaw recovery, then one-day attachment |
| MRC-5 | Carboxyl | 5,000 | 5% FBS | Three-day proliferation |

## Cell Dissociation Methods

Standard cell dissociation techniques can be used on BD PureCoat surfaces. Conditions will need to be optimized and may vary from cell type to cell type. If the cells are more difficult to remove, we recommend extending the incubation time instead of changing the concentration of dissociating agent. Accutase, TrypLE, Dispase, and 0.05% Trypsin/EDTA have been used successfully.

## Microscopy and Imaging Analysis Techniques

BD PureCoat surfaces support standard microscopy and imaging analysis techniques. For detection/other assay reagents (dyes), please follow manufacturer's instructions.

## Background

We have observed the carboxyl surface does produce an increased background when basic colormetric dyes are used (i.e. crystal violet and toluidene blue). Visualization of cells is not affected, however if you want to quantify your results, a background substraction is recommended.

In some instances where quantitative imaging of GFP-transfected cells was employed, we observed higher background fluorescence (~10%) from cells plated on BD PureCoat surfaces vs. tissue culture surfaces.

## Fixing Techniques and Immunohistochemistry

Formalin, Paraformaldehyde and Methanol has been used to fix cells.

Both single and double step immunohistochemistry has been performed and qualitative analysis reveals nothing is different from standard tissue-culture surfaces.

## Transfection assays

Lipofectamine™ 2000 is recommended as reagent for transfection experiments.

## General Guidelines for Automated Use

Optimization of automation and handling equipment conditions is recommended prior to screening. Please refer to our website for the plate maps for dimension details, or contact our Technical Service Department.

## Special Instructions for High Throughput Screening Applications

For antagonist screening, please titrate the EC70/EC90 of the agonist you use for your assay system prior to screening.

## CUSTOMER AND TECHNICAL SUPPORT

For technical assistance, contact Technical Support at:
Tel: 877.232.8995 or 978.901.7389  Fax: 978.901.7491; e-mail: Labware@bd.com

To place an order in the U.S., contact Customer Service at;
Tel: 877.232.8995  Fax: 800.325.9637 or 858.812.8889

Outside the U.S., contact your local distributor or nearest BD Biosciences office.

Visit our website www.bdbiosciences.com/discovery_labware for additional information on BD PureCoat and other Cell Culture surfaces.

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