

Corning® Roller Bottles Selection and Use Guide

Innovative Products for Biotechnology

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Introduction

In the past 90 years, animal cell culture has evolved from a laboratory curiosity into one of the leading tools of biotechnology. During this time the products used for growing cells, especially culture vessels, have also undergone an evolution. This is especially true of roller bottles.

The concept of growing cells as rotating cultures was originally developed by George Gey (1933) at Johns Hopkins University as a means of growing larger quantities of anchorage-(attachment) dependent cells. His work was primarily done in glass roller tubes. By the 1960s, much larger glass roller bottles were in common use for growing large numbers of cells, especially for viral vaccine production. Besides providing larger surface areas for growth, this culture technique has two advantages over traditional static monolayer culture: first, the gentle agitation prevents gradients from forming within the medium that may adversely affect growth; second, cells spend most of their time covered by only a thin layer of medium allowing superior gas exchange.

Corning® introduced the first sterile, ready-to-use, disposable plastic roller bottles in the 1970s. For those applications where glass roller bottles are preferred, Corning also offers a complete line of heavy duty, reusable borosilicate glass (PYREX®) roller bottles in several styles and sizes. Today, roller bottles provide a very economical means for cultivating large quantities of anchorage-dependent cells using essentially the same culture techniques as with flasks but with considerably less labor.

Corning Disposable Plastic Roller Bottles

Corning plastic roller bottles are manufactured from virgin polystyrene and feature one-piece seamless construction. The caps are manufactured from virgin, high-density polyethylene and are free of heavy metals. Both plastics meet the **USP Class VI** requirements for plastic containers and closures.

Certification Program

Corning polystyrene roller bottles are manufactured in FDA compliant and **ISO 9002** registered manufacturing facilities. Signed Certificates of Compliance are available for each lot to guarantee quality and facilitate incoming QC.

- ▶ Bottles are certified nonpyrogenic with a documented endotoxin level of less than 0.5 EU/mL to ensure consistent cell culture performance. They have been tested to meet the criteria established in the *Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Parenteral Drugs, Biological Products and Medical Devices* (FDA, 1987).
- ▶ Bottles are sterilized by gamma irradiation and dosemetrically released based on *Sterilization of Healthcare Products – Requirements for Validation and Routine Control – Radiation Sterilization*.
- ▶ Each manufacturing lot is also performance tested for product integrity. Each polystyrene bottle is then surface modified (tissue culture treated; Ramsey et al., 1984) using statistical process controls rendering it negatively charged and hydrophilic for optimal cell attachment. The bottles are then tested for cell attachment and growth using an attachment-dependent mammalian cell line in a serum-supplemented medium.
- ▶ All bottles have individually printed 8-digit lot numbers for quality assurance and to aid in product traceability. The first three digits are the Julian date, the start of manufacturing; the next two digits are the year of manufacture; and the last three digits are the run number for that year.
- ▶ Corning roller bottles are widely used in automated culture systems such as the CellMate™ Robotic Cell Culture System (<http://www.automationpartnership.com>) to produce cells for viral vaccines and high throughput drug screening programs.



Standard Corning® polystyrene roller bottles are surface modified for improved cell attachment using a traditional gas-plasma treatment. This generates highly energetic oxygen ions which graft onto the surface polystyrene chains so that the bottle surface becomes hydrophilic and negatively charged so cells can better attach. Polystyrene roller bottles are also available with a Corning CellBIND® Surface treatment that uses a novel microwave plasma process for treating the culture surface. This process improves cell attachment by incorporating significantly more oxygen into the cell culture surface than the traditional plasma treatment, rendering it more hydrophilic (wetable) and increasing the stability of the surface.

The Corning CellBIND cell culture surface:

- Improves cell attachment leading to increased cell growth and yields
- Results in more consistent and even cell attachment
- Helps adapt cells to reduced serum or serum-free conditions
- Reduces premature cell detachment from confluent cultures

Corning Smooth Wall Polystyrene Roller Bottles

The 850 cm² roller bottles are graduated to 2000 mL in 50 mL increments; the 1750 cm² bottles are graduated to 1500 mL in 50 mL increments.

These roller bottles are available with three nominal growth areas. Actual growth areas may be greater depending on the amount of medium used:

- 490 cm² (bottles are 11.12 cm in diameter, 17.30 cm in length including cap);
- 850 cm² (bottles are 11.63 cm in diameter, 27.36 cm in length including cap);
- 1750 cm² (bottles are 11.73 cm in diameter, 53.16 cm in length including cap).

Two surfaces are available:

- The standard Corning tissue culture treated surface
- The new Corning CellBIND surface treatment

Three caps are available:

- The traditional plug seal cap design is available on the 490 cm² roller bottle;
- Easy Grip caps are designed for more comfortable manual handling, have half turn on/off seating, smooth, rounded edges, and large, easy-to-grip knurls. They are not vented.
- The Easy Grip cap design is also available with filtered vents. Vent caps feature a 0.2 μm membrane sealed into the Easy Grip cap to permit sterile gas exchange when working with bottles in open cell culture systems.

Two packaging options are available:

- Packed two per clear plastic bag;
- Bulk packed 5, 20, or 22 per bag.

Corning Cat. No.	Surface Area (cm ²)	Cap Style	Surface	Graduations	Qty/ Pk	Qty/ Cs
430195	490	Plug Seal	Tissue Culture Treated	No	2	40
3907	850	Easy Grip	Corning® CellBIND® Surface	Yes	2	40
431849	850	Easy Grip	Tissue Culture Treated	Yes	2	40
431198	850	Easy Grip Vent	Tissue Culture Treated	Yes	2	40
431329	850	Easy Grip Vent	Corning CellBIND Surface	Yes	2	40
430851	850	Easy Grip	Tissue Culture Treated	Yes	5	40
431133	850	Easy Grip	Tissue Culture Treated	Yes	20	20
431344	850	Easy Grip	Corning CellBIND Surface	Yes	22	44
430699	1750	Easy Grip	Tissue Culture Treated	Yes	10	20

Corning® Expanded Surface Polystyrene Roller Bottles

Although these bottles have the same approximate outer dimensions of the 850 cm² roller bottles, the ribbed design provides twice the growth surface while maintaining the same exterior dimensions. Two smooth window areas that run the length of the bottle provide microscopic viewing as well as pouring surfaces. The bottles are graduated from 100 to 1000 mL in 100 mL increments.

Two surfaces are available:

- The standard Corning tissue culture treated surface
- The Corning CellBIND surface treatment

Two caps are available:

- Easy Grip caps are designed for more comfortable manual handling, have half turn on/off seating, smooth, rounded edges, and large, easy-to-grip knurls. They are not vented.
- The Easy Grip cap design is also available with filtered vents. Vent caps feature a 0.2 µm membrane sealed into the Easy Grip cap to permit sterile gas exchange when working with bottles in open cell culture systems.

Two packaging options are available:

- Packed two per clear plastic bag
- Bulk packed either 5 or 20 per bag

Corning Cat. No.	Surface Area (cm ²)	Cap Style	Surface	Qty/Pk	Qty/Cs
430852	1700	Easy Grip	Tissue Culture Treated	2	40
431200	1700	Easy Grip Vent	Tissue Culture Treated	2	40
430853	1700	Easy Grip	Tissue Culture Treated	5	40
431135	1700	Easy Grip	Tissue Culture Treated	20	20
431134	1700	Easy Grip	Corning CellBIND Surface	20	20
431191	1700	Easy Grip Vent	Tissue Culture Treated	20	20

Corning® PYREX® Glass Reusable Roller Bottles

Corning PYREX glass reusable roller bottles are manufactured from borosilicate glass for optical clarity and mechanical strength. They are designed to withstand repeated wet or dry sterilization.

PYREX Glass Roller Bottles with 38 mm Screw Cap

Bottles are supplied with 38 mm deep skirted, rubber-lined, phenolic screw caps.

Corning Cat. No.	Description	Cell Growth Area (cm ²)	Dimensions O.D. x Height (mm)	Qty/Cs
1400-285	Roller Bottle	840	110 x 285	2
1400-440	Roller Bottle	1170	110 x 440	2
1400-490	Roller Bottle	1330	110 x 490	2
1400-570	Roller Bottle	1585	110 x 570	2
1400-CAP	Cap, Phenolic G.P.I. 38-430	n/a	n/a	1



PYREX Glass Roller Bottles with 45 mm Screw Cap

Bottles have linerless, one-piece orange polypropylene plug seal caps with GL45 threads and drip-free pouring rings. A wide range of optional caps are available including: colored caps for ease of sorting and identification or vented membrane caps with 0.22 μm PTFE hydrophobic membranes for gas exchange.



Corning Cat. No.	Description	Cell Growth Area (cm ²)	Dimensions O.D. x Height (mm)	Qty/Cs
1425-240	Roller Bottle	680	110 x 240	2
1425-285	Roller Bottle	840	110 x 284	2
1425-370	Roller Bottle	1070	110 x 370	2
1425-570	Roller Bottle	1585	110 x 570	2

PYREX Glass Roller Bottle with 51 mm Screw Cap

Wider mouth simplifies cell harvesting. Bottles are supplied with 51 mm phenolic, rubber-lined caps.



Corning Cat. No.	Description	Cell Growth Area (cm ²)	Dimensions O.D. x Height (mm)	Qty/Cs
1450-270	Roller Bottle	670	110 x 270	2
1450-475	Roller Bottle	1330	110 x 475	2
1450-570	Roller Bottle	1585	110 x 570	2
1450-CAP	Phenolic Cap G.P.I. 51-400	n/a	n/a	1

Tips on Solving Growth Problems in Roller Bottles

The constant movement of the medium across the surface of the bottle, slow though it appears, can make it more difficult for cells to attach and grow in roller bottles compared to stationary vessels such as flasks and dishes. The constant motion of the medium can also lead to a more stressful cell environment than is found in stationary culture systems. Consequently, any technique-related issues that reduce the attachment ability of cells is magnified and clearly stands out (Freshney, 1994). This section will focus on identifying (and correcting) some of the common sources of growth problems in roller bottles. Please note that many of these growth problems are not readily observed during routine microscopic observation of live cultures. The occurrence and extent of these problems are best observed when sample cultures are first fixed and stained (1% crystal violet was used for the photos in this article) prior to observation.



Figure 1. The bottle on the left was rotated too fast resulting in uneven distribution of cells to the ends of the bottle. The bottle on the right was rotated at the correct speed. The cells in these bottles were fixed and stained with crystal violet to show their density and distribution.

Uneven Cell Attachment and Clumping

One of the most commonly encountered problems using roller bottles is difficulty getting the cells to attach and form an even monolayer in the bottle. Rotating bottles at inappropriate speeds is a common cause of attachment problems. If the bottles are rotated too quickly for cells to easily attach, areas of heavy cell growth often appear as circular bands towards both ends of the bottles (See Figure 1). This is because the medium flow is slightly slower at the ends than in the middle of the bottles. Rotating bottles too fast may also result in large clumps of cells. This results from the tendency of cells to form clumps since they find it easier to adhere to each other than to the surface of the roller bottle. Eventually these clumps become large enough that they can attach to the bottle surface. A recommended starting speed for initiating roller bottle cultures is 0.5 to 1.0 revolutions per minute (rpm) to start. However, if cells have difficulty attaching, slower speeds (0.1 to 0.4 rpm) should be used until the cells are attached.

Another possible solution to cell attachment problems in roller bottles is the Corning® CellBIND® Surface. The first novel cell culture surface treatment in over 20 years, this surface is designed to improve cell attachment under difficult conditions, such as growth roller bottles. It is also useful for growing “difficult” cells such as primary cultures or transfected cells over expressing proteins. Developed by Corning scientists, this technology uses a microwave plasma process for treating the culture surface. This process improves cell attachment by incorporating significantly more oxygen into the cell culture surface than traditional plasma or corona discharge treatments, rendering it more hydrophilic (wetter) and increasing the stability of the surface.

Unlike biological coatings, the Corning CellBIND surface is a nonbiological surface that requires no special handling or storage. Because the polymer is treated, rather than coated, the surface is more consistent and stable. This enhanced cell performance has already led to a major biotechnology company choosing Corning roller bottles with the Corning CellBIND surface for producing a new FDA approved protein therapeutic.

Table 1. Recommended Media Volumes

Corning® Plastic Roller Bottles (area)	Recommended media volumes
490 cm ²	100 to 150 mL
850 cm ²	170 to 255 mL
1700 cm ²	340 to 510 mL
1750 cm ²	350 to 525 mL

Corning PYREX® Glass Roller Bottles (area)	Recommended media volumes
670–680 cm ²	135 to 200 mL
840 cm ²	170 to 255 mL
1170 cm ²	235 to 350 mL
1330 cm ²	265 to 400 mL
1585 cm ²	315 to 475 mL
1585 cm ²	315 to 475 mL



Figure 2. Most of the cells have attached to the bottom end of this bottle as a result of it being stood on end for too long immediately after seeding it with cells.

Cell damage during subculturing, or incomplete inactivation or removal of dissociating enzymes can also make it more difficult for cells to attach and result in banding or clumping. The protein-based cell receptors used to initiate cell attachment become damaged by the dissociating procedures and must be replaced before the cells can reattach. Poorly regulated incubator temperatures (temperatures that are too high or too low) will also make it more difficult for cells to evenly attach to roller bottles.

Using too small a volume of medium, or a medium that contains insufficient attachment factors (serum-free media for example) can have very similar effects. Approximately 0.2 to 0.3 mL of medium per square centimeter of growth area is a good recommended starting volume, depending on the cell line used and the frequency of feeding (See Table 1).

It may be necessary to adjust feeding frequency (medium changes) to optimize cell growth. If the bottles are initially rotated too slowly, or if they slip or stop turning even for a short time during the initial cell attachment period, uneven longitudinal bands of cell growth may appear. Cleaning the rollers on the roller apparatus should alleviate slipping bottles. If necessary, rubber bands can be placed around the ends of the bottles to improve traction.

Bands of heavy growth at just one end of the bottle are often the result of the roller apparatus not being level. This causes an increased amount of medium and cells at the end of the bottle that is lower (see Figure 2). Furthermore, the longer it takes the cells to attach, the more time there is for them to gradually roll down the length of the bottle to the lower end before attaching. Standing a bottle on end for too long after initially seeding it with cells can have a similar effect.



Figure 3. Two clear bands caused by debris in the medium scraping away cells as the bottle was rotated.



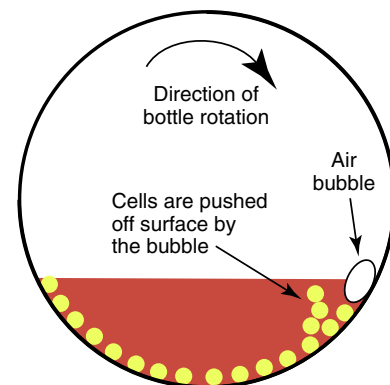
Figure 4. A clear streak free of cells resulting from cap condensation running down the cell sheet while the bottle was temporarily stored upright awaiting processing.



Figure 5. A cell monolayer damaged by scraping with a pipette (left). Cells peeling away from the surface of the bottle (right).

Clear Bands

Occasionally clear circular bands will occur on roller bottles where the cells appear to have been swept away (See Figure 3). While small pieces of rolling debris or large cell clumps can cause this to occur, one of the most common causes is the short-term presence of bubbles in the initial cell inoculum. These bubbles, when in contact with the sides of the slowly rotating bottle, can act as miniature plows, scraping off the cells as they begin to attach. Avoid bubble formation by carefully pouring medium down the sides of the bottles, or pipetting it directly into the bottom of the bottles. Cell suspensions used for inoculating roller bottles should be prepared to ensure they are bubble-free.



Streaking

Condensation (essentially pure water) falling onto exposed cells can cause some unusual patterns and events. This problem usually occurs in roller bottles that have been removed from an incubator and are standing upright at cooler room temperatures awaiting processing. Due to temperature differences, water vapor will condense on the inside of the cap. The resulting droplets may then coalesce and run down the sides of the bottle across the cells that are now only covered by a very thin film of medium. These cells will then undergo a strong osmotic shock. If they have formed a confluent monolayer, they may tear or pull apart from each other along the path the water takes, creating a visible dagger-like streak (See Figure 4). Cells that have not reached confluency may round up and float off into the medium, leaving behind a long clear streak devoid of cells.

Peeling

Heavily confluent cell monolayers (especially fibroblasts) will occasionally start to peel away from the surface of the roller bottle (See Figure 5). This often results, not from surface treatment failure, but from the formation of a flexible sheet of tightly interconnected cells and cell-manufactured extracellular matrix. Over time, mechanical stresses can develop in the cell sheet from cellular movements and contractions that may then cause the cell sheet to tear or pull away from the roller bottle. Physical damage from pipetting directly onto the cell sheet, tearing it with the end of the pipette, or other manipulations to the cell sheet may also initiate cell sheet peeling. Corning recommends trying the Corning® CellBIND® Surface if peeling occurs.

Unusual Growth Patterns

Static electrical charges that build up on roller bottles can also adversely affect cell attachment. This problem occurs more frequently when the relative humidity is very low during the winter (or year round in some lab locations). Wiping the vessels with a clean damp towel, increasing the room humidity, or using commercially available antistatic devices may eliminate or greatly reduce this problem. Extra care should be used to avoid rubbing roller bottles against the packaging when removing them from their plastic bags as this can increase the static charge.

The way in which medium is added to a roller bottle can also cause unusual growth patterns due to the differential binding of serum attachment proteins in the medium to the wall of the bottle.

Poor Growth

Many problems with the growth, maintenance, or performance of cell cultures are eventually traced back to the medium in which the cells are cultured. These medium-related problems are often slow to develop and are rarely obvious, usually requiring significant effort to track down and eliminate. Unless heavily contaminated, good culture medium is not visibly different in appearance from defective culture medium. The only good way to determine medium quality is to attempt to grow cells with it; this is the basic quality control procedure used by reliable commercial media manufacturers.

Cell cultures respond to deficient or toxic media in different ways depending upon both the nature and the degree of the problem. These responses can range from minor changes in growth rate or cell attachment, to changes in the rates of production of viruses or bioproducts, or even the total loss of the culture. Determining if the medium is responsible for a problem is relatively easy; simply test the suspected batch against a sample proven to be effective. Determining why the medium is defective is extremely difficult due to the numerous reagents and complex steps involved. Therefore, time and energy is better spent preventing media problems than trying to find and fix them later; management by prevention is the key to successful media production. The following sections will discuss three of the most common problem areas encountered using culture media.

Buffers

Many growth problems result when customers do not supply the CO₂ levels required by the bicarbonate-based buffering system of the medium they are using, resulting in poor pH control. Media buffered with low levels of sodium bicarbonate, such as found in Eagle's Minimal Essential Media (MEM) buffered with Hanks' salts (0.350 g/L sodium bicarbonate), are designed for use in sealed (air tight) culture vessels in incubators without elevated CO₂ levels. This buffer system is often used in industrial scale production using roller bottles since it does not require a CO₂ incubator. However, Eagle's MEM buffered with Earle's salts (2.2 g/L sodium bicarbonate) requires open culture vessels (dishes, plates or flasks with loose or vented caps) in a humidified incubator capable of maintaining 5% to 7% CO₂. Table 2 gives the bicarbonate levels found in some commonly used cell culture media. Usually the higher the level of sodium bicarbonate, the higher the level of CO₂ that must be supplied for optimum buffering capacity.

Table 2. Bicarbonate Levels in Some Commonly Used Mammalian Cell Culture Media

	Sodium bicarbonate levels (g/L)	Extra CO ₂ required
Eagle's Minimal Essential Medium (MEM) with Earle's salts	2.2	Yes
Eagle's MEM with Hanks' salts	0.35	No
Medium 199 with Earle's salts	2.2	Yes
Medium 199 with Hanks' salts	0.35	No
MEM α Medium	2.2	Yes
Dulbecco's Modified Eagle's Medium (DMEM)	3.7	Yes
DMEM/F12	1.2 to 2.438	Yes
Ham's F12	1.176	Yes
MCDB 131 Medium	1.176	Yes
McCoy's 5A	2.2	Yes
RPMI 1640	2.0	Yes
CMRL 1066 Medium	2.2	Yes
Leibovitz's L-15 Medium	None	No

Often, the above bicarbonate-based buffer systems are supplemented with the addition of HEPES, a widely used organic buffer. The use of this buffer can lead to additional problems upon exposure of the medium to fluorescent light (see next section below).

Fluorescent Light-induced Toxicity

The deleterious effect of fluorescent light on culture media may be the single most overlooked source of chemically induced cytotoxicity. It is very important to store media and cells growing in culture vessels in the dark away from sources of fluorescent light that will interact with light sensitive media components (riboflavin, tryptophan and HEPES). These interactions result in the production of hydrogen peroxide and free radicals that are directly toxic to cells. This well-documented problem is often ignored when there are cell growth issues (Wang, 1976, Wang and Nixon, 1978). Since the toxic effects of improperly stored media slowly increase with time, this problem is particularly difficult to identify. Besides direct cytotoxicity, other light-induced damaging effects include genetic damage (increase in mutation rates and chromosomal aberrations).

Mycoplasma

Although the problem of cell culture contamination is beyond the scope of this brochure, it is important to draw attention to another potential and widespread source of mysterious cell attachment and growth problems. (For more detailed information on the problem of cell culture contamination, refer to Lincoln and Gabridge, 1998; Ryan, 1994; Rottem and Barile, 1993; McGarrity, 1982; McGarrity, 1976.) Due to the very high densities they can achieve in cell culture (up to 10^8 /mL), mycoplasmas (unlike other contaminants such as bacteria and fungi) cause serious adverse effects on cell cultures without clouding the medium or being visible under the microscope (See Figure 6). Mycoplasmas often grow attached to the cell membrane; as a result, a single cell may have several hundred mycoplasma on its membrane which greatly affects the ability to attach and grow. An ongoing mycoplasma screening program is an essential requirement for all cell culture labs working with cell lines (Lincoln and Gabridge, 1998). Without such a program, mycoplasma contamination, along with the associated problems and embarrassment, is likely to occur at some point.

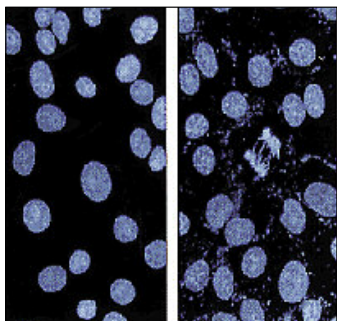


Figure 6. Photomicrographs (1000x) of VERO cells stained with Hoechst 33258 dye. DNA containing nuclei and mycoplasma stain brightly under ultraviolet light allowing the clean culture on the left (a) to easily be distinguished from infected culture on the right (b). (Photomicrographs courtesy of Bionique Testing Laboratories, Inc.)

Technical Assistance

For additional technical support and product information, please call **1.800.492.1110** or **1.978.442.2200**. Technical and product information is also available on the Corning Life Sciences web site at www.corning.com/lifesciences.

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CORNING

Corning Incorporated *Life Sciences*

Tower 2, 4th Floor
900 Chelmsford St.
Lowell, MA 01851
t 800.492.1110
t 978.442.2200
f 978.442.2476

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