Technical User's Guide

Culture of Adherent Cells Using Roller Bottles

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Introduction

Roller bottles allow scale-up of adherent cells from the 175 cm^2 growth surface in a stationary flask to 850 cm^2 in a standard roller bottle and 4200 cm^2 in an expanded surface roller bottle. This is achieved by using all of the cylindrical surface of the roller bottle for cell growth rather than just the bottom surface as in a flask. Expanded surface roller bottles have a pleated design that increases the growth surface area but retains the same outer dimensions as the standard roller bottles. Cells are bathed in medium by a constant rolling of the bottle and therefore require less media per cm^2 than what is required for static cell growth systems. Moreover, dynamic systems, such as a roller bottle, often generate higher cell densities per surface area than static systems. Therefore a higher titer of cells or cellular products can be achieved.

The cellular environment of the roller bottle is very dynamic compared to static growth systems. Adherent cells are constantly exposed to alternating liquid and gas phases, providing efficient equilibration of gases and dilution of metabolites. Roller bottles are generally used as a closed cell culture system rather than an open CO_2 dependent system typical of most static cell culture. Therefore, sparging thoroughly with 5% CO_2 prior to bottle closure and maintaining sufficient headspace in the bottles may be beneficial, depending on the medium buffer condition. In addition, because of the higher cell density per media volume, roller bottle cultures must be monitored for acidification of the media caused by the generation of metabolites and therefore may require media replacement.

Basic procedure for roller bottle culture

1. Standard cell culture media and reagents can be used. The use of CO_2-dependent media may require pre-gassing of the cultures with 5 - 10% CO_2 prior to incubation or the addition of organic (i.e. 5-20 mM HEPES (pH = 7.2-7.4)).

2. Complete medium is added to the bottle at approximately 1mL per 5 cm^2 of growth surface area. The volume of the head space in the bottles should be at least 5 times greater than the media volume to ensure an adequate amount of CO_2 for carbonate buffering systems and O_2 for respiration. If an expanded surface roller bottle is used, lower volumes that sufficiently cover the side and pleats of the bottle may provide a more concentrated cellular product, however, earlier feeding or harvesting times may be required to prevent the exhaustion of medium nutrients.

3. Add the number of cells by either an established split ratio or per cm^2 of growth surface area. Typical seeding concentrations for cell lines are 1 x 10^3 to 1 x 10^5 cells/cm^2. It is important to note that seeding concentrations will be dependent upon cell line used and cultivation culture conditions, e.g. nutrient concentrations, serum used, serum percentage used and media volume used to name a few. Thus, the number of cells to be plated in an expanded surface roller bottle should be about twice the number in a standard bottle based on the cell growth surface area. Ideally, the best method for determining the most optimal seeding density is to perform growth curves at different seeding concentrations.

4. Tighten the bottle closure and place in the roller apparatus.

5. The bottles should be rotated at 0.5-1.0 rotations per minute (rpm) for the cell attachment phase. This usually requires 24 - 48 hours depending on the cell line. A slower rotation speed (0.3-0.5 rpm) may be necessary for cells that adhere poorly.

6. Cells can be harvested with a cell disassociation liquid such as trypsin or EDTA. Rinse the roller bottle two times with phosphate buffered saline (PBS) and add enough trypsin to cover entire length and pleats of the bottle. Place the roller bottles back in the roller apparatus and rotate 0.3-0.5 rpm until the cells detach. This usually requires 5 - 10 minutes. Cell detachment should be monitored by microscopic examination.

7. To efficiently remove suspended cells following cell disassociation hold the bottle at a 45° angle and pour. If using an expanded surface bottle, rotate the bottle to drain all the fluid from the pleats. The cell suspension can also be removed from the base of the bottle with a pipette or other mechanical methods.

Tips for roller bottle use

Cell growth can be monitored using an inverted microscope that allows the light source to be raised to accommodate the roller bottle. Cells in the expanded surface bottles can be viewed along viewing panels.

The increased surface area of the expanded surface roller bottle permits culture of 4 times as many cells without expanding capital or labor needs.

PETG can be cut with relative ease for full access to the growth surface. A razor type utility knife with a solid handle or a hot knife can be used.

Roller bottles may be coated in the usual manner for cell culture if a biological or other type coating is required.

It is recommended that medium be pre-warmed to the temperature of incubation before addition to bottles and sealing of closure. The addition of cold media to the bottles prior to incubation at 37°C may cause an increase in pressure and the bloating of the bottle. Although this will not lead to breakage, it may affect the consistency of cell plating.

Thermo Scientific™ Nunc™ Roller Bottles			
	Surface Area, cm^2	**Complete Media Volume Recommendation (0.2-0.4mL/cm^2)***	**Cell Disassociation Recommendation (mL) Trypsin or EDTA**
Smooth Surface	850 cm^2	170-425	20
	1050 cm^2	210-420	20
	1800 cm^2	360-720	40
Expanded Surface	1450 cm^2	290-580	50
	1700 cm^2	340-680	60
	2100 cm^2	420-840	60
	4200 cm^2	840-1680	120

* 0.2mL/cm^2 is a typical volume. More or less media may used. Media volumes will be dependent on cell type, media type (DMEM, MEM, Serum Free, etc)

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