PBS-MINI System

Application Guideline

This document is intended to serve as a guideline detailing the best practices for operating the PBS-MINI as it applies to cell culture processing with emphasis on cell therapy applications. The topics below are categorized according to how a user might design and perform a cell culture run.

Designing run conditions:

1) Working volume

The PBS-MINI has working volume specifications of 20-100 mL in the PBS-0.1 and 100-500 mL in the PBS-0.5, a 5-fold turndown ratio in each size.

Cell Culture Volume Range:
Since higher shear stress may be imparted by the system in low working volumes when the wheel impeller protrudes from the liquid surface, our recommended working volume range during cell culture for most applications is 60-100 mL in the PBS-0.1 and 300-500 mL in the PBS-0.5.

Cell Harvest Volume Range:
The lower end of the working volume ranges is intended for use during *in situ* harvesting when higher shear stress may be required to dissociate cell clusters into single cells or remove cells from microcarriers.

2) Agitation rate

The agitation rate is process-specific, and it is determined empirically for each process. The settling velocity of cells within cell culture medium depends on whether they are cultured in single cell suspension, in clusters of various sizes, or attached to scaffolding such as microcarriers, which come in various sizes and densities.

Generally we recommend applying the lowest agitation rate that results in full/ near-full suspension of cell clusters or cell-bound microcarriers. Low shear stress is believed to be conducive to cell growth, especially for primary cells that have not been adapted to grow in suspension and may not be as shear resistant as some of the industrial cell lines that have been developed for protein production.

For each process, we recommend that the agitation rate be gradually increased until full/ near-full suspension of cell clusters or cell-bound microcarriers is obtained, which is typically in the range of 15-25 RPM in both the PBS-0.1 and PBS-0.5. For cells that experience difficulty adhering to microcarriers in suspension culture, intermittent agitation (i.e. cycles of on-off agitation) over a 12-24 hour period may promote cell attachment initially, after which the agitation can be set to mix continuously.

The cell culture should also be monitored daily to check for the size of cell clusters or cell-bound microcarriers. The agitation rate may need to be adjusted according to the desired goal of your process. Some processes might favor large cell clump formation to maximize cell yield, while others might favor maintaining a reasonable cell cluster size to achieve high cell viability and homogeneity.

3) Microcarrier type (if applicable)

There are various types of microcarriers commercially available, and they vary in material of construction, size, and coating type. Selection of microcarrier type is process-specific and depends on cell type and the goals of process performance. Pall Life Sciences (Solohill brand), GE Healthcare Life Sciences, and Corning are among the popular suppliers, and specific information on their microcarriers can be found on each manufacturer's website.

Although PBS Biotech does not have any preference among the various types of microcarriers, we have the most experience working with Solohill polystyrene microcarriers of 125-212 micron diameter range with collagen coating for culturing human mesenchymal stem cells (hMSCs). If there is regulatory concern with using collagen, however, the Corning Low Concentration Synthemax II microcarriers that utilize synthetic, non animal-derived coating also work well with hMSCs.

4) Microcarrier and cell concentrations at seeding (if applicable)

The concentration of microcarriers used depends on the target seeding density and the desired ratio of cell per microcarrier at seeding. Generally, to allow sufficient space for cell expansion on the surface of microcarriers, we recommend that the ratio of cell per microcarrier at seeding be kept reasonably low (less than 5 cell/ microcarrier). Greater

than 1 cell/ microcarrier loading is recommended to ensure high microcarrier colonization percentage (percentage of microcarriers with at least 1 attached cell).

As an example, 16 g/L concentration of the Solohill collagen-coated microcarriers at 2 cell/ microcarrier seeding (~14,700 cell/mL) has worked well in our hands for culturing hMSCs.

Performing a cell culture run:

5) Medium addition

Cell culture medium and other liquid components may be poured or pipetted into a MINI vessel through the open port after removing the vent cap. The PBS-0.5 is equipped with two ports, but the user may choose the port that is more convenient to use for liquid handling without impacting vessel functionality.

Although the vessels have graduation marks in 20% increments of full working volume, we recommend that volume of each component be accounted for by more accurate methods (either measured out ahead of time in a transfer bottle or by graduation on a pipette).

Medium and other components may be warmed in a water bath prior to transfer into a MINI vessel or equilibrated in an incubator once transferred into the vessel.

6) Cell inoculation

The seed culture is added to a MINI vessel through the open port after removing the vent cap. To minimize shearing effects from splashing, it is recommended that the seed culture be pipetted into the vessel sub-surface or just above the liquid surface instead of being poured in.

7) Sampling

A sample is removed from a MINI vessel through the vent port after removing the cap. It is recommended that a pipette be used to remove a sample. If the size of cell clusters

or microcarrier aggregates is large, then it is recommended that you use either a pipette with a larger tip or break the tip off of a pipette (only up to 10 mL pipette).

For large cell clusters or cell-bound microcarriers that have a fast settling velocity, it is recommended that the vessel agitation be maintained while a sample is removed to allow for a homogeneous sample to be taken. Setting up a separate MINI base unit in the biosafety cabinet could facilitate this step.

A minimum recommended volume for a representative sample is 5 mL in both the PBS-0.1 and PBS-0.5. Larger sample volumes may be required for large cell clusters or microcarrier aggregates. A 10 mL pipette is an ideal size to use for most sampling application.

8) Medium exchange

Medium exchange is performed in a MINI vessel by first turning off agitation on its MINI base unit to allow the cell clusters or cell-bound microcarriers to settle to the bottom of the vessel.

To prevent the Vertical-Wheel® impeller in the vessel from turning inadvertently and causing the cells to re-suspend, it is recommended that the vessel be situated on a base unit during medium exchange. This allows the magnets on the Vertical-Wheel impeller to couple with the magnets on the MagDrive of the base unit, thereby locking it in place.

Cell-free medium may then be removed by pipetting, with care taken to prevent cell removal or inadvertent re-suspension of cells. Once the desired volume of cell-free medium is removed, fresh medium should be pipetted in, per Section 5 above.

9) Cell harvest

- If cell dissociation is to be performed outside of a MINI vessel, the entire vessel content should be removed by pouring or pipetting into a sterile container before cell washing and dissociation steps are initiated.

- For *in situ* harvest where cell dissociation is performed within the MINI vessel, cell-free medium should first be removed per Section 8 above. If cells must be washed prior to dissociation (e.g. for removing serum), then sufficient amount of phosphate-buffered saline or equivalent solution should be added to the vessel per Section 5 and removed per Section 8, with the steps repeated as required by the process.

  An appropriate amount of trypsin, TrypLE ™ enzyme, or other cell dissociation agent should then be pipetted into the vessel before the vessel is returned to a base unit in the incubator for an appropriate duration of time necessary for dissociation. It may be necessary to use a higher agitation rate than was used during the culturing phase to help detach the cells. Afterwards, a quenching solution (e.g. serum-containing medium) to deactivate the cell dissociation agent activity should be added to the vessel per Section 5 above.

  - For a microcarrier-free process, the entire content of the MINI vessel should then be transferred to a separate container by pouring or pipetting, for additional wash steps with centrifugation.

  - For a microcarrier process, only the cells should then be collected from the MINI vessel by allowing the microcarriers to settle to the bottom of the vessel and pipetting out the microcarrier-free cells, similar to Section 8 above, prior to additional wash steps with centrifugation. To expedite this process of separating out the microcarriers from cells, the vessel content may also be poured through a sterile strainer of appropriate mesh size (70-micron mesh is commonly used for 125-212 micron microcarriers). The strainer would retain the microcarriers while allowing single cells to pass through into a collection tube for further processing.

Please feel free to contact the technical support team at PBS Biotech if you have any questions.

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