

## Perfusion: hardware needs

Perfusion is a cell culture process in which cells are retained in the bioreactor with a continuous exchange of medium. This process removes cell waste and spent medium while constantly replenishing nutrients and carbon sources with fresh medium. In this white paper, we will cover the hardware that is necessary for the perfusion process: cell retention mechanisms, pumps, and equipment to address intensification challenges.

### Cell retention mechanisms

Cell retention mechanisms keep cells in the bioreactor during a perfusion cell culture process. There are two types of filtration methods for cell retention: alternating tangential flow (ATF) and tangential flow filtration (TFF). In ATF, the flow of medium from the reactor is drawn through a hollow fiber membrane and then pushed back into the reactor. Pores are sized to allow spent medium to pass through the hollow fiber membrane while retaining cells. Spent medium is then pulled off for downstream processing at a rate significantly slower than the cell-containing medium being cycled between the reactor and cell retention mechanism through the hollow fibers. TFF is similar to ATF except that medium from the reactor is cycled through the hollow fiber membrane in one path and requires a separate path to return cells to the bioreactor.

There also are settling methods, such as passive settling, acoustic settling, and centrifugation. In passive settling, separation of spent medium from the cells is attained by gravity. Very slow flow rates are required to allow the slightly heavier cells to settle away from the spent medium.



Figure 1. Types of pumps used for perfusion operations.

The cell-heavy medium is then cycled back to the reactor, and the cell-light spent medium is pulled off for further processing downstream. In acoustic settling, sound waves are used to aggregate cells. These sound waves accelerate cell settling, allowing for better separation at higher flow rates than passive settling. For centrifuge-based approaches, centrifugal acceleration is used to rapidly settle and separate cells from spent medium. All settling methods are imperfect, causing some amount of cells lost into the spent medium to be removed from the system. This may require additional clarification steps to the spent medium prior to the separations workflow.

### Pumps

Pumps are an integral component of the perfusion process and are used for:

- Medium flow into a bioreactor
- Recirculation between a bioreactor and cell retention device
- Spent medium removal
- Excess cell removal, also known as a bleed

A peristaltic pump is typically used for medium flow into the bioreactor. The pump is often paired with a flow meter or a scale to improve flow rate accuracy.

The type of pump used for recirculation is partially determined by the cell retention device. For ATF, a simple diaphragm pump is usually the most practical since it natively generates a low-shear bidirectional flow action (Figure 1). For all other cell retention devices, a recirculation flow with low shear is desired to reduce risk of cell damage. Due to lower operational shear, a centrifugal pump or a quaternary diaphragm pump is preferred over a peristaltic pump.

Spent medium removal can use a peristaltic pump; however, when using a filter-based cell retention mechanism, it is desirable to have consistent pressure across the filter membrane. Therefore, a quaternary diaphragm pump, an offset roller dual-head peristaltic pump, or other device between a pump and the filter membrane to reduce pressure oscillation may be optimal. The spent medium pump is often controlled via level control to maintain consistent volume within the bioreactor. For some types of perfusion operations, especially continuous perfusion, a bleed pump, typically a peristaltic pump, may be necessary to maintain cell density control. The bleed pump may be automated using a capacitance probe or autosampler to run the bleed pump via a viable cell density (VCD)-feedback control loop.

## Intensification challenges

For high cell density operations, existing equipment should be evaluated to meet increased performance demands. The mass flow controller (MFC) should be able to handle peak gassing demand that occurs from increased mass transfer ( $k_L a$ ) needs. Increased mass transfer may require increasing the gas flow rate of your  $O_2$  MFC. The MFC should also be capable of smooth operation during the initial lower gassing demand. In some cases, having two  $O_2$  MFCs is the best solution to address such a large dynamic range of gassing demand (Figure 2).

In a perfusion process, the increased viscosity and  $k_L a$  demand may require higher mixing power, often scaled in terms of power input to volume (PIV) measured in watts per cubic meter. Higher mixing power can be achieved by increasing RPM, impeller size, type, or quantity.

Shear may be an important concern depending on the cell clone. Altering impeller size, quantity, and type may allow for higher mixing PIV without greatly increasing mixing shear.

In order to achieve a smooth perfusion process, the right hardware must be used. Without a clear understanding of each component discussed, there can be a risk of not achieving an optimal process.

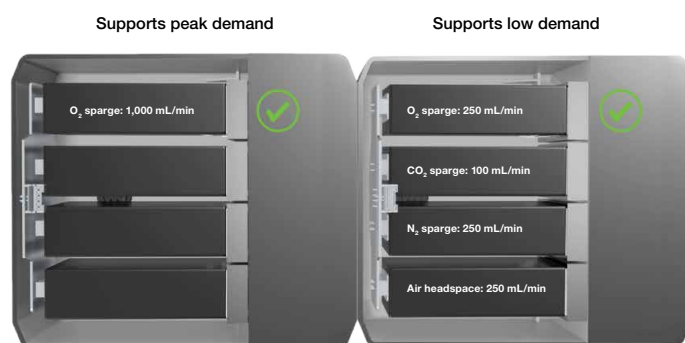


Figure 2. Illustration of two MFCs to support a range of gassing requirements.

Contact your sales representative or get more information at [thermofisher.com/perfusion](https://thermofisher.com/perfusion)