Application Note

Mixing Modes in IRO® for CAR-T Manufacturing

Overview

IRO® is a next generation platform that automates and standardizes critical steps in the cell and gene therapy manufacturing workflow. At its core is a single-use Bioreactor with novel geometry, featuring a compressible cylindrical sidewall (bellows), a circular moving base for agitation, and a circular baffle. These design elements allow the Bioreactor to support a wide range of hydrodynamic environments, from static (similar to a T-flask) to fully turbulent (similar to a stirred tank reactor), while also offering enhanced control over cell suspension. Using a flexible mixing protocol, tailored to the needs of the cells at each stage of the process, i.e. activation, transduction, expansion; significantly improves biological performance demonstrated by enhanced cell growth and transduction efficiency. This application note outlines the different mixing modes in the bellows Bioreactor, and the role of each in a standard CAR-T manufacturing process.

Summary

- IRO® Bioreactor has novel bellows-based geometry for flexible mixing.
- Three agitation modes, tailored to different stages of the manufacturing process: Static, Rock and Compression Mixing.
- Summary of each mode and its role in a standard CAR-T manufacturing process.
- Flexible operating parameters and their definitions.
- Key insights for generating flexible mixing, fine-tuned to the process.

IRO Mixing Modes

The Bioreactor operates in three distinct mixing modes, each suited for different phases and media volumes within a standard CAR-T manufacturing workflow.

Static Mixing

The Bioreactor base remains static, with mixing only occurring through diffusion. This mode is comparable to the operating principles of a T-flask and is best suited for smaller working volumes at the beginning of the process.

Rock Mixing

The Bioreactor base oscillates to an angle ($\pm \alpha°$) around the central horizontal axis, as illustrated in Figure 1. This generates wave motion, which enhances oxygen mass transfer through the surface and drives gentle convective mixing in the liquid. This mode is suitable for moderate working volumes and is best applied during the activation and transduction phases of the CAR-T process.

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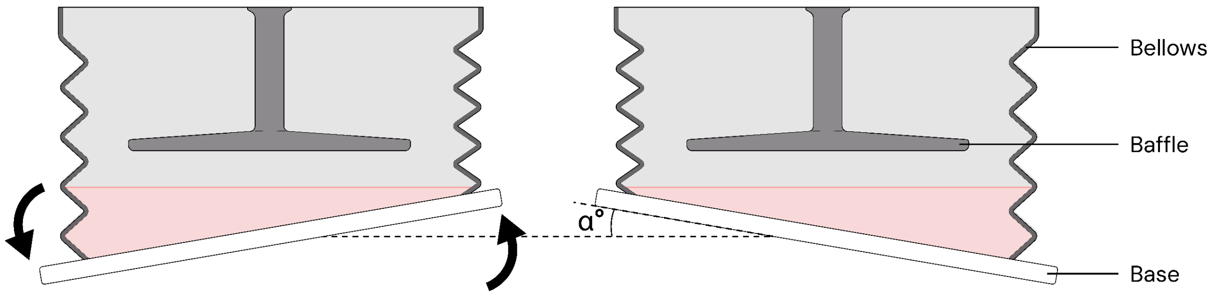

Figure 1. Schematic of IRO Bioreactor in Rock Mixing Mode. The Bioreactor base oscillates to an angle (±α°) around the central horizontal axis (shown as a dashed horizontal line).

Compression Mixing

The Bioreactor base oscillates between a low and a high position, with stroke length (S mm), relative to its starting (low) position, compressing the Bioreactor bellows as illustrated in Figure 2. In this mode, superior potential for mixing and oxygen mass transfer is achievable, which can support larger working volumes and higher cell densities.

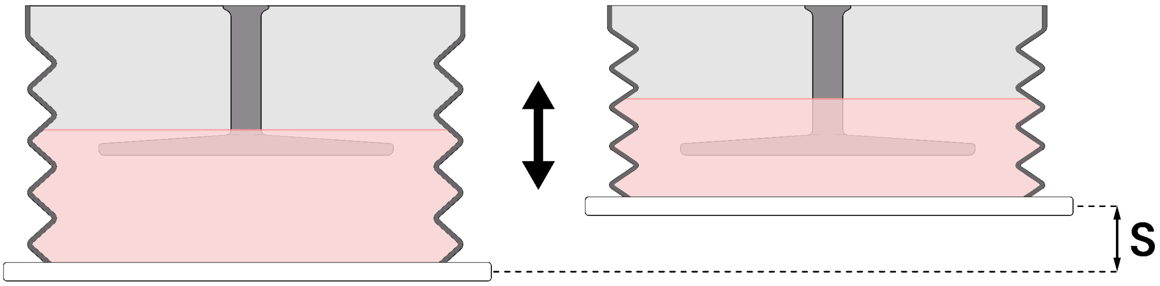

Figure 2. Schematic of the IRO Bioreactor in Compression Mixing Mode. The Bioreactor oscillates up and down with a stroke length (S mm) defined relative to the distance in mm between the low and high position, causing the bellows to compress.

Flexible Operating Parameters for IRO Mixing Modes:

Operating Parameter	Description
Agitation Rate [rpm]	The number of rock or compression cycles the Bioreactor completes per minute.
Rock Angle [°]	During Rock Mixing, the inclination of the Bioreactor base with respect to the horizontal axis.
Stroke Length [mm]	During Compression Mixing, the vertical distance the Bioreactor base travels from its starting position (low) to its maximum position (high).
Base Height [mm]	The starting position of the Bioreactor base at the beginning of either Rock or Compression Mixing, relative to the lowest possible position.

About Us: Ori Biotech is a London and Philadelphia-based manufacturing technology company pioneering flexible process discovery with seamless translation and scalable commercialization of cell and gene therapies.