# Utility Of The Mobius® CellReady 3 L Single-Use Bioreactor
# For Upstream Process Development Applications

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## Abstract

Single-use processing solutions have become an important trend in the biopharmaceutical industry spanning both upstream and downstream applications. The advantages of the single-use approach to industrial cell culture versus traditional stainless steel and/or glass bioreactors has resulted in the recent commercialization of several single-use bioreactors (SUBs) at small (3 L-15 L), intermediate (50-500 L) and large scales (>1000 L). Merck Millipore has recently introduced the Mobius CellReady 3 L bioreactor, a SUB that shares the features of conventional bioreactors but offering ease-of-use benefits associated with single-use technology that was developed for the optimization of mammalian cell growth and expression. Detailed characterization of this vessel, both in terms of function for CHO cell growth and expression and volumetric mass transfer coefficients ($k_La$) for oxygen, as compared to glass bioreactors, was performed. The results demonstrate similar cell culture performance and $k_La$ values. Based on these results, we have been able to streamline our cell culture work by using the 3 L CellReady bioreactors to scale up inoculum (i.e., seed-train) for large scale bioreactor experiments. By pooling the cultures from 2-3 small scale single-use bioreactors, sufficient biomass was readily achieved to inoculate directly into the 200 L CellReady Bioreactor. There, a 40 L n-1 step preceded the 200 L production experiments. Subsequently, the 3 L CellReady bioreactors were used to monitor performance of the 200 L large-scale cell culture experiments as satellite bioreactors. Tracking of cell culture performance in this fashion was both effective and convenient. In addition to its use in process characterization and development work, this study demonstrated that the small size and portability of the 3 L CellReady bioreactor make it an ideal platform for both seed-train and satellite applications.

FIGURE 1. **Single-Use Bioreactors Simplify Large-Scale Bioreactor Runs.** When used together, the Mobius CellReady 3 L and 200 L Bioreactors provide a completely single-use solution for cell culture experiments.

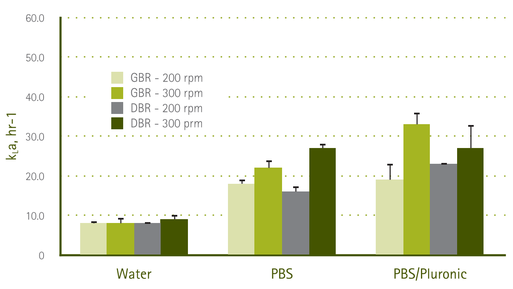

## Characterization of the Mobius CellReady 3 L Bioreactor

### BIOREACTOR DESIGN COMPARISON

| | CellReady 3 L (DBR) | Applikon® 3 L (GBR) |
|---|---|---|
| **Impeller** | • Larger diameter<br>• Marine scoping low shear | • Smaller diameter<br>• Steeper-angled flat blades |
| **Microsparger** | • 15 – 30 micron polyethylene<br>• Bottom of vessel | • 15 micron sintered steel<br>• Centered below the impeller |
| **Open Pipe Sparger** | • Single-hole<br>• Bottom of vessel | • 7-hole L sparger<br>• Centered below the impeller |

## Results

### BUFFER EFFECTS ON $k_La$ VALUES

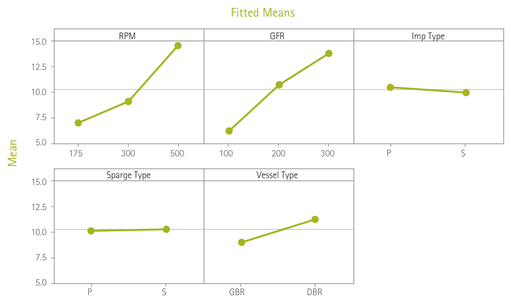



**Method**

The $k_La$ was determined via the static gassing out method for both 3 L CellReady and Applikon® 3 L glass bioreactors.

- Glass vessel were fitted with Applikon's standard axial flow impeller and a 15 micron microsparger
- The dissolved oxygen probe was initially calibrated to 100% at air saturation and brought to < 2 % with nitrogen before each experiment
- Sparge rate: 0.1 vvm with air; no overlay (2 L working volume)
- Each experiment was performed in 37 °C water, PBS or PBS/Pluronic® + antifoam; n=3.

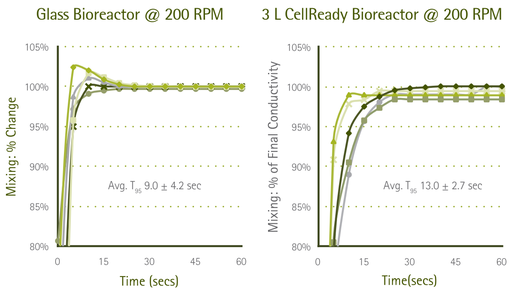
### $K_La$ DETERMINATIONS (200, 300 RPM)




**Method**

- Main effects determined and plotted Minitab® Statistical Analysis Software
- Impeller type and sparger type had no impact on $k_La$ levels
- Agitation and gas flow rates had significant impacts on $k_La$, as expected
- Vessel type impacted $k_La$; CellReady had positive effect
  *Possible explanations:*
  - Off-centered placement of sparger below impeller
  - Sparger-impeller distance & placement
  - Location of DO probe relative to sparger/impeller

### DETAILED $K_La$ MAPPING




**Conductivity**

- Range: 10-50 mS/cm
- measured at 5 sec intervals and 37 °C
- Values normalized to maximum value

**$T_{90}$ mixing time**

- < 10 seconds in both vessels

**Conclusions**

- Impeller designs contribute subtle differences in conductivity profiles between vessels
- Statistically insignificant difference in $T_{95}$ mixing times under the conditions tested

## Cell Culture Performance

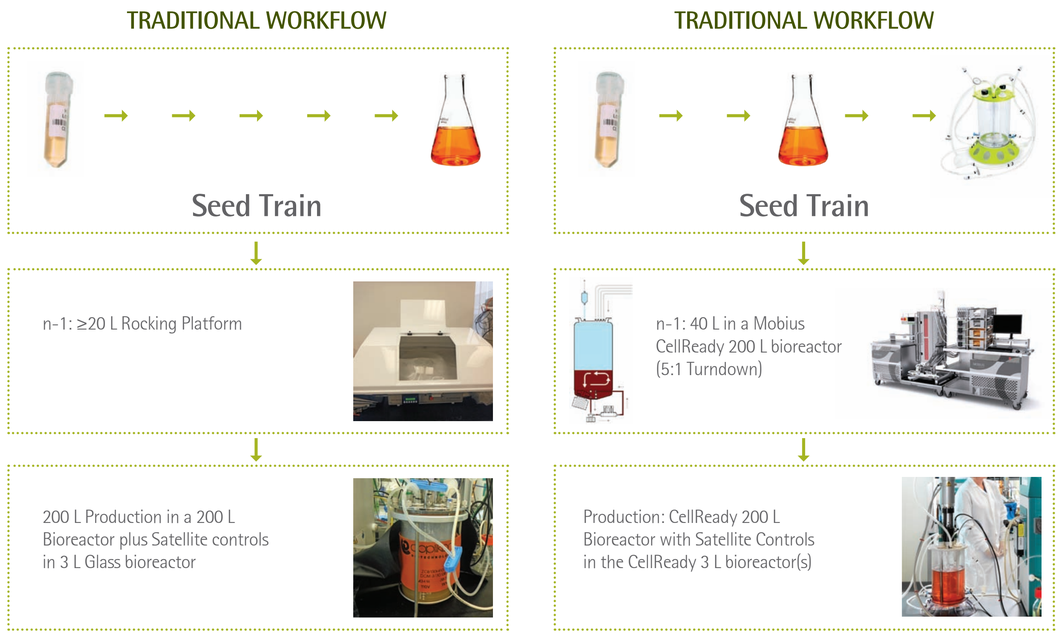



FIGURE 2: **(A) An Example of a Traditional Workflow for a 200 L Cell Culture Run and (B) an All-Single-use Workflow Using the CellReady 3 L Bioreactor.** Combining the ease-of-use of the CellReady 3 L single-use bioreactor with the low working volume of the CellReady 200 L Bioreactor, a completely single-use process can be developed. Since there is no need for complicated assembly, CIP, or SIP, users save time and simplify their process from seed train through production.

### TABLE 1

The process parameters for seed train runs through production are outlined in Table 1. The CellReady 3 L bioreactors were controlled with Applikon EZ-Controllers and the CellReady 200 L bioreactor was controlled with a Finesse RDPD system.

| Operating Parameters | Set Point Values 3 L | Set Point Values 200 L |
|---|---|---|
| Agitation Rate | 200 rpm | 83 rpm |
| Sparger Type | polyethylene microsparger | polyethylene microsparger |
| Temperature | 37° C | 37° C |
| DO | 30 % | 30 % |
| pH | 6.95 | 7 |
| Flow rate of SensorReady pump | NA | 3 L/min |
| Seeding Density | 2 x $10^5$ cells/mL | 2 x $10^5$ cells/mL |

## Viable Cell Density

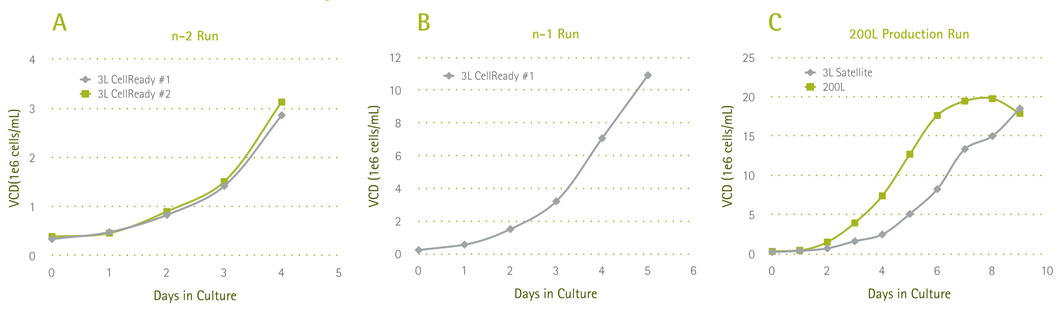



FIGURE 3: **Viable Cell Density (A) n-2 Run (B) n-1 Run and (C) 200 L Production Run.** Bioreactors were run in batch mode and sampled daily. Cell number was determined using a Vi-CELL™ XR (Beckman Coulter). After 4 days in culture, the two CellReady 3 L single-use bioreactors yielded enough biomass to inoculate a 40 L n-1 culture in the Mobius CellReady 200 L bioreactor. After 5 days, the cell density in the 200 L single-use bioreactor was reduced to 2 x $10^5$ cells/mL and the volume increased to 200 L with the addition of fresh media. There was a short lag in the growth of the 3 L satellite due to a temperature excursion during the run.

## Viability


FIGURE 4: **Viability (A) n-2 Run (B) n-1 Run and (C) 200 L Production Run.** Bioreactors were run in batch mode, sampled daily and viability was determined using a Vi-CELL XR (Beckman Coulter). At each step, the cell viabilities remained consistently high. The low cell viabilities on day 1 of each run can be attributed to the presence of antifoam in the media, which is known to impair the measurement of viability.

## Conclusions

- Mobius CellReady 3 L single-use bioreactors represent a convenient alternative to traditional methods used to seed the 40 L n-1 cultures for large-scale, production runs. This platform offers pH and DO control from seed through production.
- Three liter single-use bioreactors can also be used as satellite bioreactors to monitor the process in the 200 L bioreactor.
- The use of the 3 L single-use bioreactor reduces the amount of media needed for seed train. By using the same platform for seed train and satellites, it is possible to simplify the equipment requirements as the same controllers and process parameters can be used to meet the needs of both functions.

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