Engineering characterization of the single-use Xcellerex XDR-500 stirred-tank bioreactor system

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CY13920-27May20-AN

Engineering characterization of the single-use Xcellerex™ XDR-500 stirred-tank bioreactor system

This application note describes the physical characteristics of the XDR-500 bioreactor system suitable for use in mammalian cell culture applications. The presented data can be useful in process transfer and comparison of different bioreactor systems.

Introduction

For both conventional and single-use bioreactor systems, the knowledge of physical parameters such as k_La, mixing time, and power input is important for transferring of processes between different types of bioreactors. Characterization data is also useful in determination of a system's suitability for intended applications. The single-use XDR-500 stirred-tank bioreactor system can be used for various cell culture applications. The aim of this study was to give a detailed description of the physical characteristics of XDR-500 in terms of mixing time, heating and cooling times, power input, and k_La when equipped with a single-use XDA 500 L cell culture bag assembly.

Materials and methods

System setup

The XDR-500 system was equipped with an air-cooled temperature control unit (TCU) with 9 kW heating and 1.5 HP cooling capacity. A single-use XDA 500 L cell culture Pro bag assembly with an integral pitched-blade impeller and eight discs of micro spargers with 2 µm pore size was used for determination of mixing time, heating and cooling times, power input, and k_La. The exhaust filter of the bioreactor was equipped with the included filter heater. For temperature measurements, a standard XDR resistance temperature detector (RTD) was used.

Mixing time

Mixing time was evaluated by determining the time required to reach 95% of a pH step change (t_{m95}) (Fig 1). The XDA 500 L cell culture Pro bag was filled with phosphate buffered saline (PBS). The pH shift was generated by adding acid (0.5 M HCl in PBS) in one shot from the top of the bioreactor into the liquid at a ratio of 1:1000 to the liquid volume. The pH was recorded using nine external pH probes positioned in the bioreactor as depicted in Figure 2. To establish starting conditions, base (0.5 M NaOH in PBS) was added at a ratio of 1:1000 to the liquid volume after each mixing time experiment. Bioreactor content, corresponding to the volume of added acid and base, was removed from the bulk liquid after each test run to ensure constant test volume. Mixing time experiments were run at three different volume and agitation rate settings: 100, 300, 500 L and 30, 115, and 200 rpm, respectively. The temperature was controlled at 37°C, and the impeller was run counterclockwise to provide an upward fluid flow.

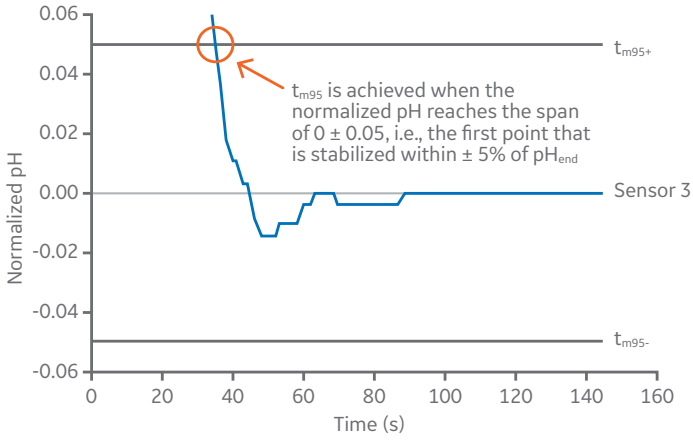

Fig 1. Example of normalized pH data for t_{m95} determination. The gray lines indicate the interval where t_{m95} is achieved.

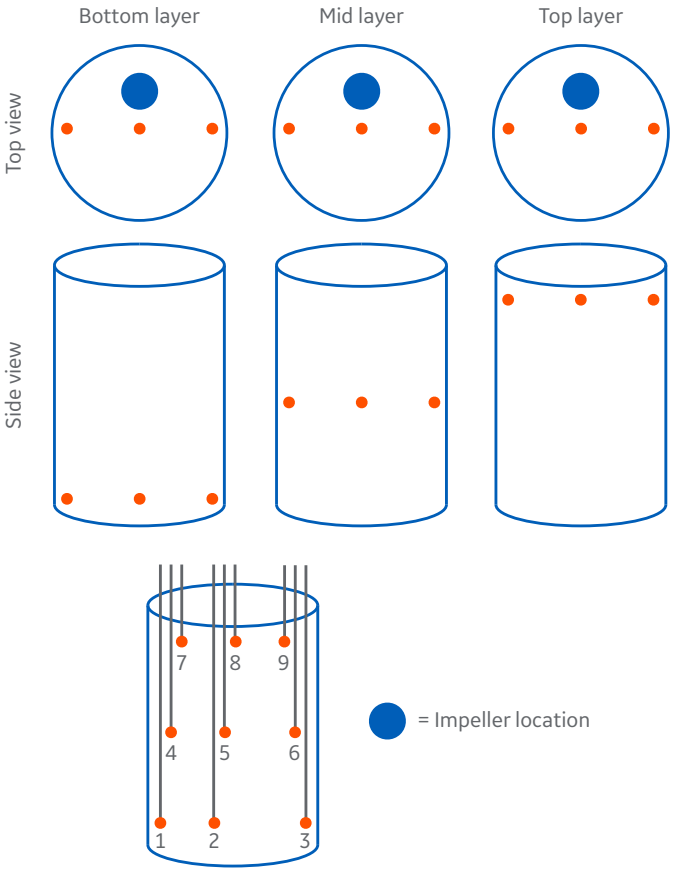

Fig 2. The pH probe positions at the maximum working volume (500 L). At 300 L, the mid and top probe layers were lowered proportionally with the fluid to capture the three levels within the smaller volume. At 100 L, the pH probes of the top and mid sections were removed and the pH was recorded by using three probes in one level. Acid and base were added from the top as close to the center as possible for all experiments.

Heating and cooling

The heating-cooling response was assessed by calculating the time to reach 95% of the temperature step change (t_{95}), for the heating intervals 5°C to 20°C, 20°C to 37°C, and 5°C to 37°C and for the cooling intervals 37°C to 5°C, 37°C to 20°C, and 20°C to 5°C. Testing was performed for three different working volumes: 100, 300, and 500 L. The XDA 500 L cell culture bag was filled with a saline solution consisting of 6 g/L of NaCl dissolved in purified water. The upward fluid flow was held constant at an agitation rate of 150 rpm, as agitation would not affect heating time significantly above a certain threshold. The vessel temperature control PID parameters were set in accordance with factory default settings (P = 4, I = 30, D = 0, DB = 0).

Power input

Power input to the XDR-500 system was assessed by measuring the motor current at agitation rates between 40 and 240 rpm (upward fluid flow) under gassed (10 L/min) and ungassed conditions. The XDA 500 L cell culture bag was filled with 6 g/L NaCl, 1 g/L poloxamer 188, and 50 ppm active silicone (Antifoam C, Sigma) in purified water to a volume of 500 L. Temperature was controlled at 37°C. The motor current was recorded from the servo drive of the XDR-500 system and converted into torque using the torque constant of 2.04 (± 10%) Nm/A (Kollmorgen, Radford, VA, USA). To compensate for the power loss due to friction in the motor and impeller assembly, power was measured during zero load conditions for each tested agitation setting. The zero load condition was established by wetting the impeller bearing without submerging the impeller blades.

Power input (P) was calculated from the motor current readings using the following equation:

[Eq. 1]: $$P = (I_{load} - I_{zero\ load}) * K_T * 2\pi N$$

To determine the power number (N_P), the following equation was used:

[Eq. 2]: $$N_p = \frac{P_o}{N^3 * D_i^5 * \rho}$$

Reynolds number (N_{Re}) was calculated using the following equation:

[Eq. 3]: $$N_{Re} = \frac{N * D_i^2 * \rho}{\mu}$$

Where:

P = power input in W

I_{load} = motor current at load conditions in A

$I_{zero\ load}$ = motor current at zero load conditions in A

K_T = bioreactor's torque constant in Nm/A

P_0 = power input in non-aerated liquid in W

N = impeller speed in s^{-1}

D_i = impeller diameter in m (0.264 m for XDR-500)

ρ = medium density in kg/m^3 (here 1000 kg/m^3 was used)

μ = medium viscosity in kg/m/s (here 0.001 kg/m/s was used)

Volumetric oxygen transfer coefficient (k_La)

The experiments were set up in accordance with design of experiments (DoE), using a Box–Behnken design where volume, agitation, and air flow rate were altered to varying levels. Before experiments were initiated, the dissolved oxygen (DO) sensor was calibrated. For the k_La measurements, the XDA bag was filled with purified water supplemented with 6 g/L NaCl, 1 g/L poloxamer 188, and 50 ppm active silicone (Antifoam C, Sigma). Testing was performed at three liquid volumes of 100, 300, and 500 L, and the temperature was controlled at 37°C to simulate typical culture conditions. Agitation was varied between 40, 120, and 200 rpm in the up-flow direction. Air flow rate was varied between three levels: 1, 10.5, and 20 L/min. To measure DO, a standard XDR DO sensor (Mettler Toledo) was used. All eight sparger discs with 2 µm pore size were tested simultaneously in each experiment. The oxygen was depleted from the liquid by addition of nitrogen gas, and the DO response was recorded from the time when air flow and agitation had been started. From the recorded DO data, the k_La coefficient could be determined by plotting $\ln(DO^* - DO_t)$ as a function of time $(t - t_0)$, for which the negative slope yielded the k_La coefficient according to the following equation:

[Eq. 4]: $\ln(DO^* - DO_t) = -k_La(t - t_0) + \ln(DO^* - DO_{t0})$

Where:

DO^* = DO value in equilibrium with the gas bubble concentration, that is, the stabilized value after the measurement is finished.

DO_t = DO value at time t.

t_0 = time when the measurement is started.

DO_{t0} = DO value when the measurement is started.

After finalizing the experiments, the obtained k_La coefficients were modeled in the DoE software, MODDE™ version 11.0.0.1717 (Umetrics AB), giving the possibility to assess k_La values for any volume, agitation, and air flow setting within the tested ranges.

Results

Mixing time

In Figure 3, results from the mixing time experiments are shown. Only data from the probe position generating the longest t_{m95} for each run is plotted to display the worst-case scenario. The shortest t_{m95} was determined to 12 s when measured at 100 L and 200 rpm, whereas the longest t_{m95} was determined to 192 s when measured at 500 L and 30 rpm. Figure 4 displays the t_{m95} (average of duplicate experiments) for each probe position for experiments performed at maximum working volume (500 L) to show how the mixing time varies depending on position in the bioreactor.

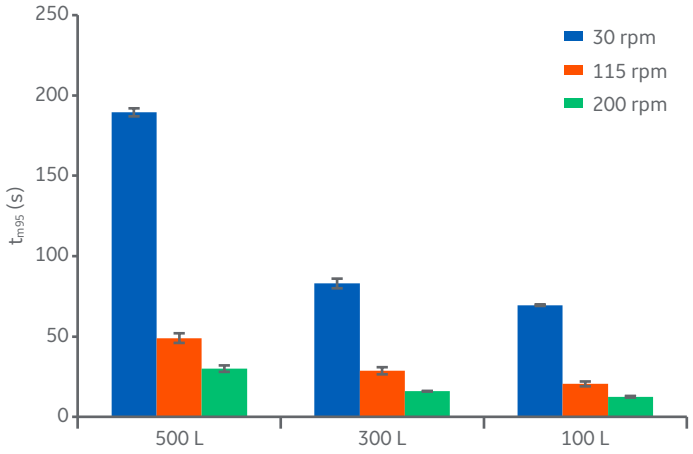

Fig 3. Average mixing times (t_{m95}) from the probe positions that generated the longest mixing time for all tested volumes and agitation rates. Error bars correspond to one standard deviation.

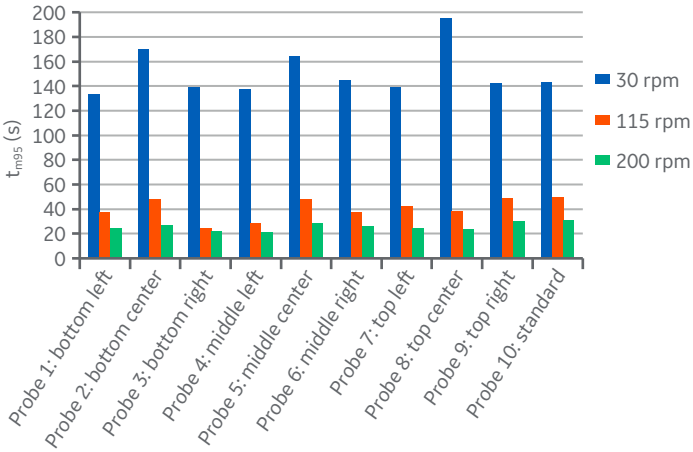

Fig 4. Mixing time (t_{m95}) for each probe position for maximum working volume (500 L). Results are averaged values from duplicate runs.

Heating and cooling

The results from the heating and cooling experiments are shown in Figure 5. The shortest time for temperature change was achieved for heating from 5°C to 20°C, with heating times of less than 1.5 h for all volumes tested. Cooling the liquid from 37°C to 5°C required the longest time, between 3 and 7 h depending on the volume. In general, the time required for heating and cooling was found to increase with liquid volume.

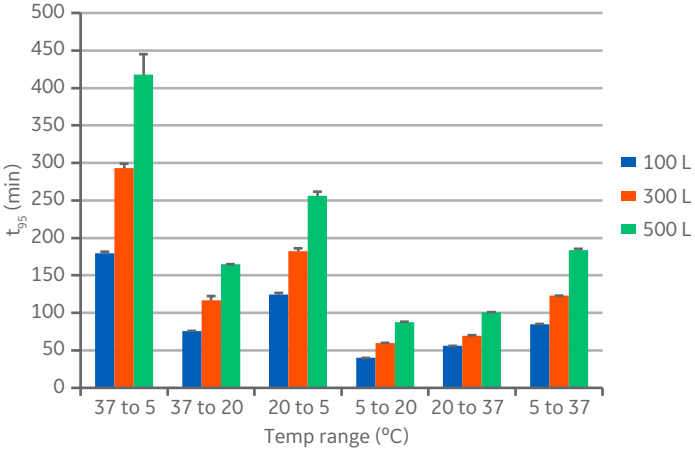

Fig 5. Results from the heating-cooling experiments. Error bars correspond to one standard deviation.

Power input

Figure 6 shows volumetric power input at maximum working volume (500 L) plotted against agitation rate for gassed (10 L/min) and ungassed conditions. The power input was found marginally lower for gassed conditions compared with ungassed conditions, with an average P_g/P_0 corresponding to 0.88. In Figure 7, calculated power number from experimental power input data is plotted against Reynolds number. At agitation rates above 130 rpm, the calculated power number remains nearly constant with an average value of approximately 0.98.

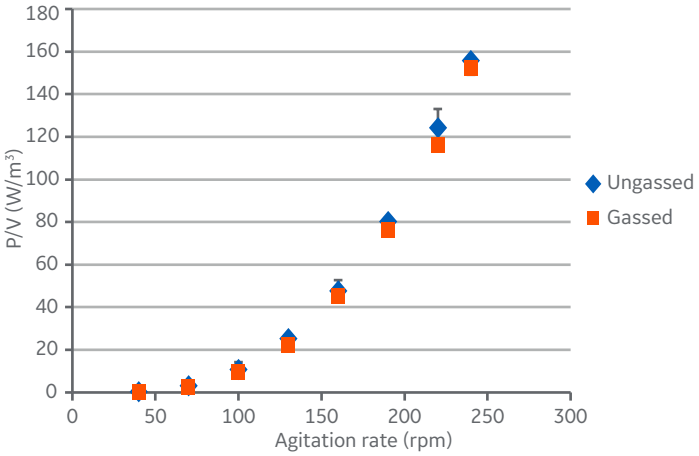

Fig 6. Power input per liquid volume plotted against agitation rate at 500 L working volume. The air flow was 10 L/min for gassed conditions. Error bars correspond to one standard deviation.

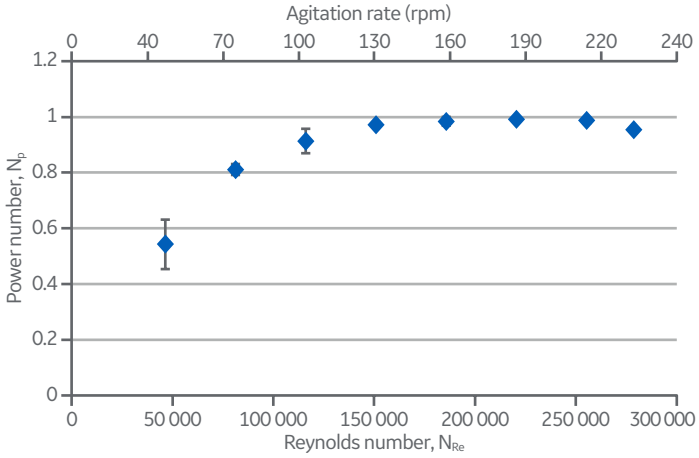

Fig 7. Power and Reynolds numbers calculated from the measurements shown in Figure 6. Error bars correspond to one standard deviation.

Volumetric oxygen transfer coefficient (k_La)

Results from k_La determination are shown in Figure 8 (nonmodeled k_La data plotted in bar graphs) and Figure 9 (contour plot generated by the DoE software from experimental data). Measured k_La values ranged from 4.3 to 72.8 h^{-1}. The k_La value was found to increase with agitation and air flow rate. Increasing the liquid volume had a negative impact on k_La. Data from one experiment performed at high agitation in low volume, 100 L, which is a condition out of range for most cell cultures, was excluded from the DoE model as it would have skewed the overall fit. Hence, the contour plots displayed for 100 L in Figure 9 might underestimate the k_La value to some extent. For the DoE model, the condition of combining high gas flow and high agitation at the maximum volume was not included. However, the k_La was also estimated for 500 L at 200 rpm and a gas flow rate of 30 L/min, which yielded a k_La of approximately 40 h^{-1}.

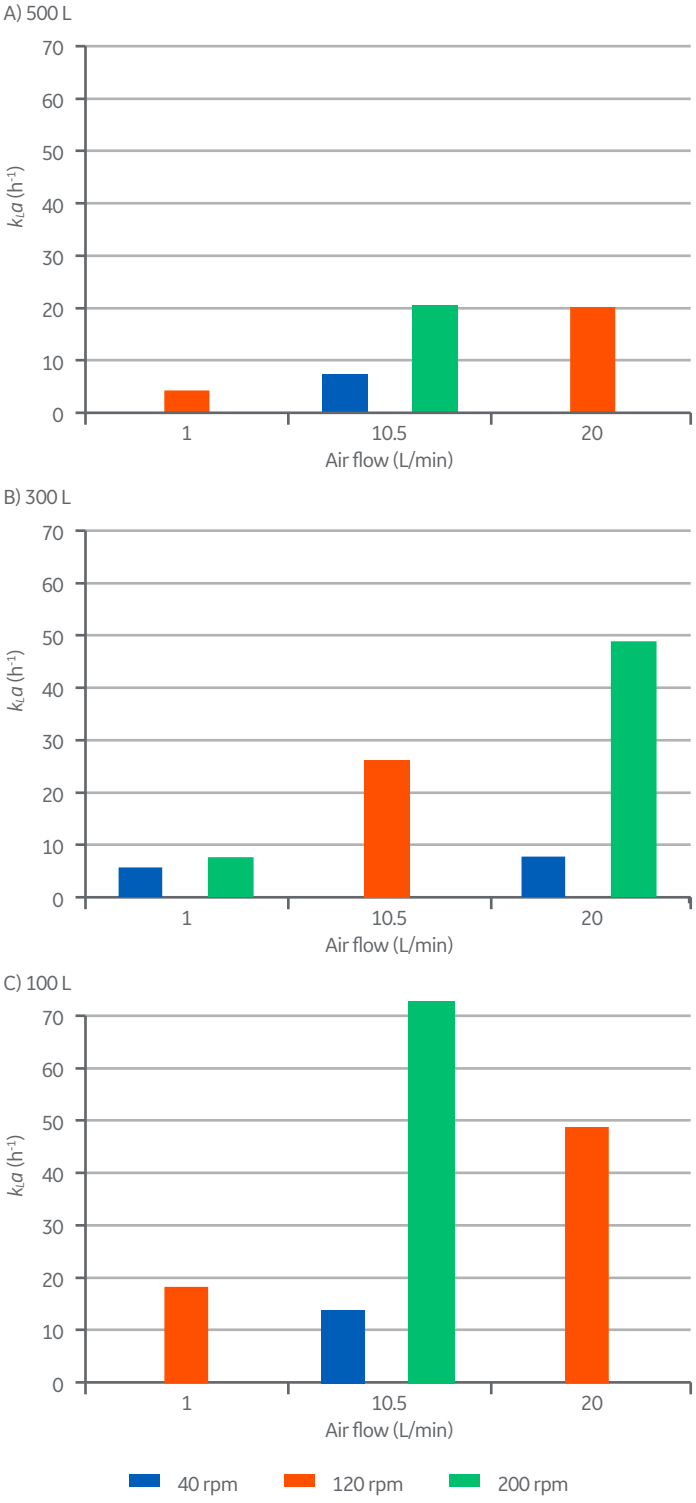

Fig 8. Results from the k_La tests for the 2 µm sparger in (A) 500 L, (B) 300 L, and (C) 100 L.

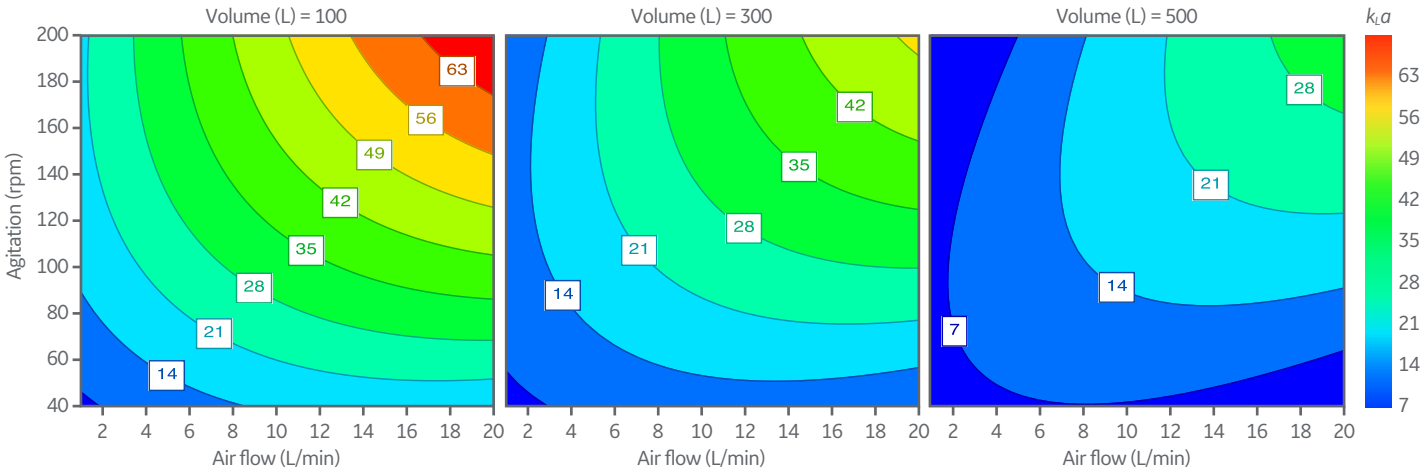

Fig 9. 4D response contour plot displaying k_La obtained with the 2 µm sparger pore size at varying air flow, volume, and agitation. The model fit (R^2) is 0.995, the model predictability (Q^2) is 0.93, and the residual standard deviation (RSD) of the model is 1.68.

Conclusions

This application note gives a detailed description of the physical characteristics of the XDR-500 stirred-tank bioreactor system in terms of mixing time, heating and cooling times, power input, and k_La. Mixing time results show sufficient mixing capacity to ensure a well-mixed tank. Heating and cooling capability, power input, and volumetric oxygen transfer coefficients were determined within ranges typical for culture applications, and obtained values were found to meet the requirements of commonly used cell lines. The generated information can be used for process transfer, scale-up, and comparison of different bioreactor types.

Disclaimer

The results from the characterization experiments and the conclusions presented in this application note are valid for this specific study. Other study conditions could have significant impact on the outcome. For each parameter, certain variability in the results can be expected depending on choice of method, measuring equipment, and test conditions such as temperature and liquid composition.

Ordering information

Product	Description	Product code
XDA-500 Pro bioreactor bag	500 L, pitch blade impeller, and eight discs of micro spargers (2 µm)	888-0070-C

To order the XDR-500 system, please contact your local sales representative.

gelifesciences.com/bioprocess

TR29321412, TR29324346

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752 USA
GE Healthcare Europe, GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Japan Corporation, Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073 Japan
GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA UK
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