SARTORIUS

Application Note #16

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Rapid and Scalable Media Preparation With Single-Use Magnetic Mixing Systems

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Executive Summary

This application note presents a new approach to increase the speed and consistency of media preparation steps in bio manufacturing. The method combines ready to use media formulations from SAFC®, with scalable, high efficiency, single-use mixing systems provided by Sartorius Stedim Biotech. The mixing system will be part component of Flexact® MP, a configurable disposable solution for media preparation.

Two examples of large volume media preparation steps Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media are presented. The contained transfer of powdered media formulations into the high torque single-use mixing system enables a rapid dissolution and dispersion of the media powders in liquid for volumes of 50 L to 1,000 L. The performances of the single-use mixing system are characterized with quantitative (conductivity measurement) and qualitative (visual inspection) techniques. The proposed method and system provide seamless scale-up and consistent rapid media mixing for process development and GMP manufacturing.

Introduction

This application study presents the performances of a fully single-use mixing solution for the large scale preparation of two different media. The mixing technology selected for this application is Flexel® for Magnetic Mixer[1] with volumes of 50 L, 200 L and 1000 L. The magnetic coupling of the impeller with the Magnetic Mixer Drive Unit enables a rotation speed up to 300 rpm, providing a powerful mixing of the media.

The first medium tested in this study is the Ex-Cell™ EBx® GRO-I Serum-Free for Embryonic Stem Cells. This medium is an animalcomponent free, serum-free dry powder formulated for the growth of EB66® cells. The EB66® cell line is proprietary to Vivalis (Saint-Herblain, France). The EB66® cell line is a fully characterized duck cell line utilized in cell-based vaccine manufacturing and for the production of recombinant viral vectors and therapeutic recombinant proteins.

The second medium tested on this study is the EX-CELL™ CD CHO Fusion. It is a chemically defined, animal-component free medium developed for the long-term growth of Chinese Hamster Ovary (CHO) cells. The absence of any large macromolecules allows for isolation and purification of secreted proteins from the cells. This medium is supplied without L-glutamine to aid in media stability, to avoid L-glutamine degradation that causes ammonia build-up and to provide an appropriate medium for the culture of CHO cells using the Glutamine Synthetase, or GS, System™. This medium does not contain hypoxanthine or thymidine to allow for its usewith dihydrofolate reductase (DHFR-) gene amplification systems.

Purpose of the Application Study

The purpose of this application study is to assess the performances of the Flexel® for Magnetic Mixer technology to dissolve the two media.

The mixing times are determined by conductivity and visual inspection of the solution in the Flexel® Bag for Magnetic Mixer.

Materials and Methods

The list of materials and equipments used for this application is:

1. Standard Flexel® Bag for Magnetic Mixer
   (50 L: FMB114867, 200 L: FMB114893, 1000 L: FMB114896)
2. Powder Transfer Bag System
   (15 L: FMA114008, 30 L: FMA114009)
3. Palletank® for Lev Mixer and Magnetic Mixer
   (50 L: FXC110820, 200 L: FXC110821, 1000 L: FXC113384)
4. Magnetic Mixer Drive Unit, 230V, EU power cord
   (ref. LT-DU-006-EU)
5. Powder Bag holder 200-400-650 L (ref. FXA114344)
6. SAFC® media:
   - Ex-Cell™ EBx® GRO-I
     (Product number: 24530C/44076)
   - EX-CELL™ CD CHO Fusion
     (Product number: 25365C/44075)
7. Sodium Bicarbonate (SAFC®: 90421C)
8. NaOH (1M)
9. HCl (1M)
10. Conductivity sensor: WTW InoLab Cond 740i
11. pH sensor : Knick SE 101
12. Floor scale: Sartorius IF S4 1500RR-1

[1]This product uses Pall patended Magnetic Mixer technology.
All information on patents can be found at www.Pall.com/patents.

Method Used:

1. The Flexel® Bag for Magnetic Mixer is placed into the Palletank® with conductivity and pH sensors.
2. The bag is filled with deionised water to 80% of the final volume (water temperature: 20 °C)
3. The mixing speed is turned on and set up to the maximum speed of 300 rpm to optimize powders dispersion
4. Media powders are added slowly through the top port to ease the powder incorporation into the water
   - Ex-Cell™ EBx® GRO-I : final concentration 19.06 g/L
   - EX-CELL™ CD CHO Fusion: final concentration 20.09 g/L
5. When the media is dissolved, sodium bicarbonate is added:
   - final concentration : 1.6 g/L for Ex-Cell™ EBx® GRO-I
   - final concentration: 1.25 g/L for Ex-Cell™ CD CHO Fusion

The powders are incorporated in the Flexel® Bag for Magnetic Mixer using either:

- SAFC® bucket liner
- or Sartorius Stedim Biotech Powder Transfer Bag for a contained transfer to the mixing bag assembly.

6. pH is adjusted by using NaOH or HCl:
   - to 6.9 – 7.1 for the Ex-Cell™ EBx® GRO-I
   - to 7.2 - 7.4 for the EX-CELL™ CD CHO Fusion
7. Deionised water is added to achieve the final volume
8. The medium is filtered (step not done during the study)
9. Sampling and QC testing on sampling according to SAFC procedures

pH and conductivity measurement are collected to illustrate process steps and show mixing performances:

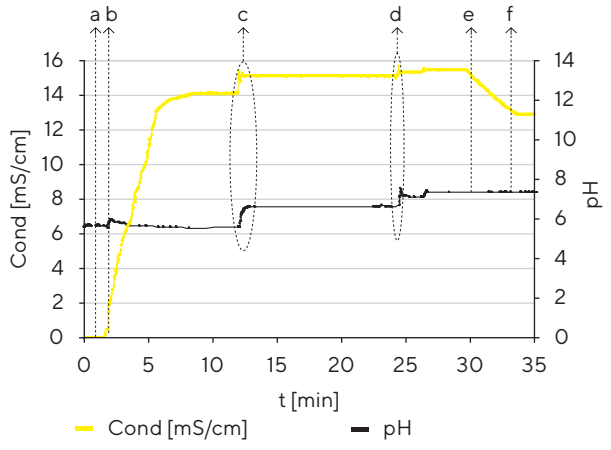


Fig. 1: Media preparation process steps

a: end of water filling (deionised water is added to achieve 80% of the final volume)
b: start mixing and media powder addition
c: start sodium bicarbonate addition
d: pH adjustment when needed
e: start final dilution (Deionised water is added to achieve the final volume)
f: end of final dilution

9. Two mixing times are monitored from the addition of media powders:
   9.1 "mixing time 1" is determined from the conductivity signal as follows:
   The "mixing time 1" corresponds to the time when 95% of the final value is reached and when all next measurements stay within a 5% tolerance.

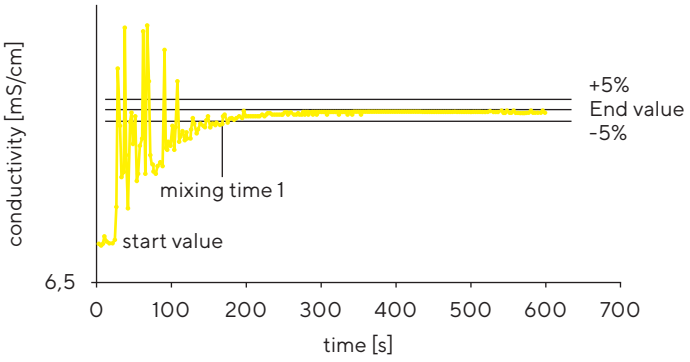


Fig. 2: General principle of mixing time determination via conductivity

9.2 "mixing time 2" is determined by a visual inspection.
The "mixing time 2" corresponds to the time when all suspended particles are visually dissolved.

200 L Flexel® with Magnetic Mixer Technology

200 L Palletank® for Magnetic Mixer equipped with the Powder Transfer Bag System for the mixing trial

Results and Discussions

1. Mixing Performances Results

Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media have been prepared in Flexel® Bag for Magnetic Mixer at 50 L, 200 L and 1000 L scales. Mixing time's results are presented for each media at the different scales.

All the results show clearly the high performance of the Flexel® Bag for Magnetic Mixer to prepare both media formulations.

Mixing time results for Ex-Cell™ CD CHO Fusion – 50 L
For the Ex-Cell™ CD CHO Fusion preparation at 50 L, the media powder was added in less than 1 minute by using SAFC® bucket liners. The media powder was mixed in less than 2 minutes (visual check). During the preparation, a pH adjustment was needed. Finally the Ex-Cell™ CD CHO Fusion preparation at 50 L took less than 15 minutes.

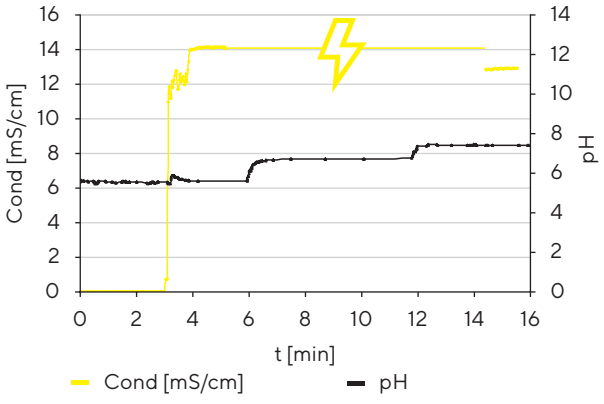


Figure 3:
Ex-Cell™ CD CHO Fusion preparation in 50 L Flexel® Bag for Magnetic Mixer

⚠ Note: conductivity value not stable at 50 L due to the presence of air bubbles around the conductitivity cell (strong vortex). A stable value of the conductivity could be observed only at lower impeller rotation speed.

Mixing time results for Ex-Cell™ CD CHO Fusion – 200 L
For the Ex-Cell™ CD CHO Fusion preparation at 200 L, the media powder was added in less than 2 minutes by using Sartorius Stedim Biotech Powder Transfer Bag System. The media powder was mixed in less than 1 minute according to conductivity and in less than 4 minutes according to visual check. During the preparation, a pH adjustment was needed. Finally the Ex-Cell™ CD CHO Fusion preparation at 200 L took less than 15 minutes.

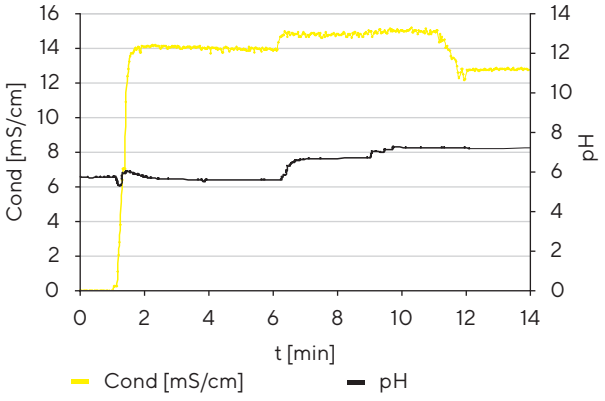


Figure 4:
Ex-Cell™ CD CHO Fusion preparation in 200 L Flexel® Bag for Magnetic Mixer

Mixing time results for Ex-Cell™ CD CHO Fusion – 1000 L
For the Ex-Cell™ CD CHO Fusion preparation at 1000 L, the media powder was added in less than 2 minutes by using SAFC® bucket liners. The media powder was mixed in less than 5 minutes according to conductivity and in less than 10 minutes according to visual check. During the preparation, a pH adjustment was needed. Finally the Ex-Cell™ CD CHO Fusion preparation at 1000 L took less than 40 minutes.

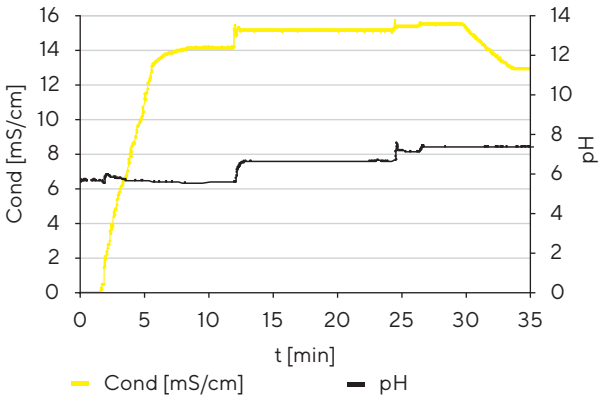


Figure 5:
Ex-Cell™ CD CHO Fusion preparation in 1000 L Flexel® Bag for Magnetic Mixer

Mixing time results for Ex-Cell™ EBx® GRO-I -50 L

For the Ex-Cell™ EBx® GRO-I preparation at 50 L, the media powder was added in less than 1 minute by using SAFC® bucket liners. The media powder was mixed in less than 4 minutes (visual check). During the preparation, a pH adjustment was not needed. Finally the Ex-Cell™ EBx® GRO-I preparation at 50 L took less than 15 minutes.

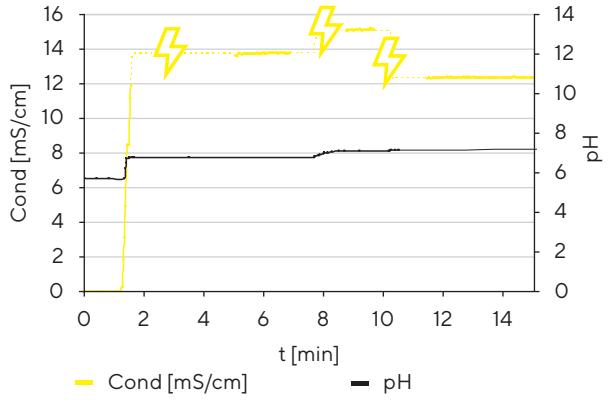


Figure 6:

Ex-Cell™ EBx® GRO-I preparation in 50 L Flexel® Bag for Magnetic Mixer

⚠ Note: conductivity value not stable at 50 L due to the presence of air bubbles around the conductitivity cell (strong vortex). A stable value of the conductivity could be observed only at lower impeller rotation speed.

Mixing time results for Ex-Cell™ EBx® GRO-I -200 L

For the Ex-Cell™ EBx® GRO-I preparation at 200 L, the media powder was added in less than 1 minute by using Sartorius Stedim Biotech Powder Transfer Bag System. The media powder was mixed in less than 1 minute according to conductivity and in less than 4 minutes according to visual check. During the preparation, a pH adjustment was not needed. Finally the Ex-Cell™ EBx® GRO-I preparation at 200 L took less than 15 minutes.

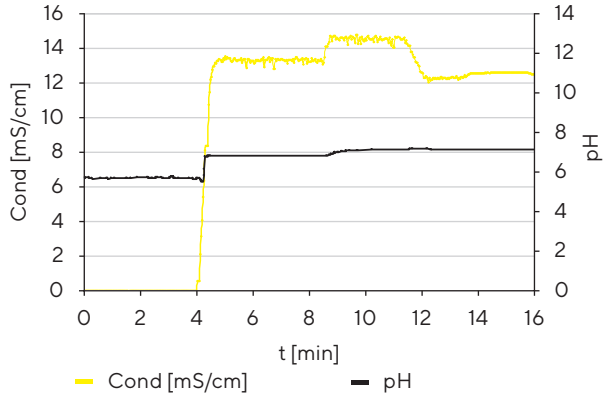


Figure 7:

Ex-Cell™ EBx® GRO-I preparation in 200 L Flexel® Bag for Magnetic Mixer

Mixing time results for Ex-Cell™ EBx® GRO-I -1000 L

For the Ex-Cell™ EBx® GRO-I preparation at 1000 L, the media powder was added in less than 3 minutes by using SAFC® bucket liners. The media powder was mixed in less than 5 minutes according to conductivity and in less than 15 minutes according to visual check. During the preparation, a pH adjustment was not needed. Finally the Ex-Cell™ EBx® GRO-I preparation at 1000 L took less than 45 minutes.

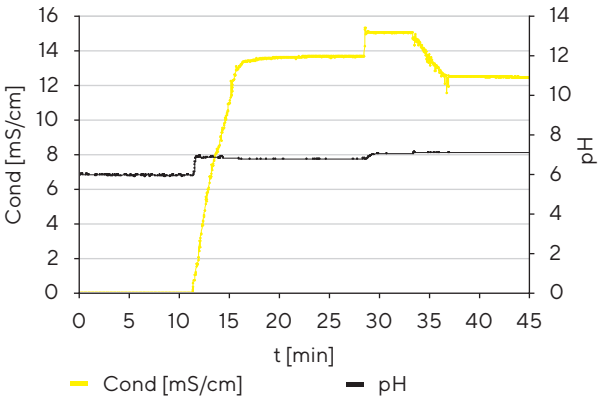


Figure 8:

Ex-Cell™ EBx® GRO-I preparation in 1000 L Flexel® Bag for Magnetic Mixer

Quality Control Results:

After media preparation and filtration, some samplings are taken for both media solutions and several quality control tests are performed to confirm the quality of the product.

Figures 9 and 10 show that the QC tests results done on each media are conform.

Ex-Cell™ CD CHO Fusion

Test description	Results	Status
Osmololity	309	Conform
pH	7.3	Conform
Growth Promotion | Cytotoxicity (Specification: CD >/= 2.0 × 10E6 cells/ml	CD = 3.1 × 10E6 cells/ml	Conform

Figure 9: Ex-Cell™ CD CHO Fusion – Quality control results

Ex-Cell™ EBx® GRO-I

Test description	Results	Status
Osmololity	286	Conform
pH	7.5	Conform
Growth Promotion (Specification: Doubling time </= 21 hrs each pass)	15.6 hrs	Conform

Figure 10: Ex-Cell™ EBx® GRO-I - Quality control results

General Comments:

The mixing times reported in this study include the transfer time of the multiple Sartorius Stedim Biotech Powder Transfer Bags System (for the 200 L scale experiment) or SAFC® bucket liners (50 and 1000 L experiments) into the mixing bag assembly.

A rapid dissolution was observed for both Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media due to the strong mixing torque at 300 rpm.

For the 50 L volume, the vortex at 300 rpm in the 50 L bag volume resulted in the generation of air bubbles that interfered with conductivity measurement with air trapped in the conductivity cell. The conductivity was not unwavering even though mixing was completed. A stable value of the conductivity could be observed only at lower impeller rotation speed. Therefore mixing time cannot be precisely defined using the conductivity signal. The mixing time is at least as good as with the larger scale and the different process step could be followed by the pH monitoring.

For the 200 L and 1000 L volume, the conductivity of the solutions reaches a stable value in few minutes. However, some fine particulates can still be visually observed in the solution. The agitation at 300 rpm was maintained until the particulates became visually totally dissolved. This visual control is facilitated by the large windows of the Palletank®.

Powder Transfer Bag: Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media are fine powders. During both preparations, some dust appears during the media powder introduction into the Flexel® Bag for Magnetic Mixer. The use of Powder Transfer Bag System docked onto the Flexel® Bag show clearly the advantage to maintain a high containment and reduce the exposure of operator to chemicals.

In this study, a 15 L Powder Transfer Bag was used for the 200 L Ex-Cell™ CD CHO Fusion preparation and a 30 L Powder Transfer Bag System was used for the 200 L Ex-Cell™ EBx® GRO-I preparation.

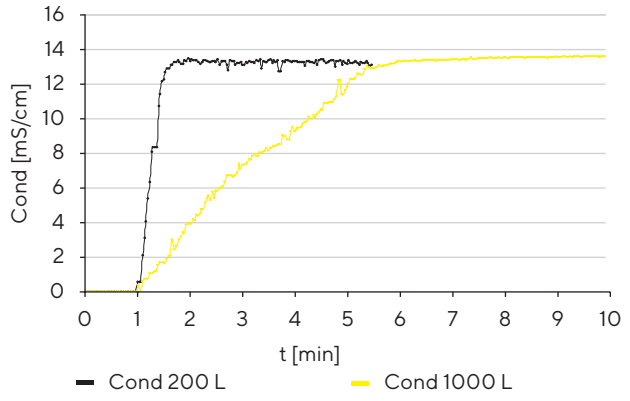
During Ex-Cell™ CD CHO Fusion and Ex-Cell™ EBx® GRO-I media a preparation, some foam appears on the top during the media powders dissolving. The foam formation could be reduced by adding slowly the powder. Also the Flexel® Bag for Magnetic Mixer could be designed with a higher bag chamber volume to allow enough head space and therefore optimize the handling.

2. Mixing Performance vs. Volume of Media

The mixing performances versus the volume media is compared only on the media powder dissolution step. In fact, the others additions (sodium bicarbonate and acid | base if needed) are almost instantaneous.

Even if the mixing time increases as expected with the volume, the media dissolution is still rapid for the 1000 L scale experiment. The stable plateau conductivity is reached in less than 10 minutes (based on conductivity measurement) for both media.

Ex-Cell™ EBx® GRO-I -200 vs. 1000 L



Ex-Cell™ CD CHO Fusion – 200 vs. 1000 L

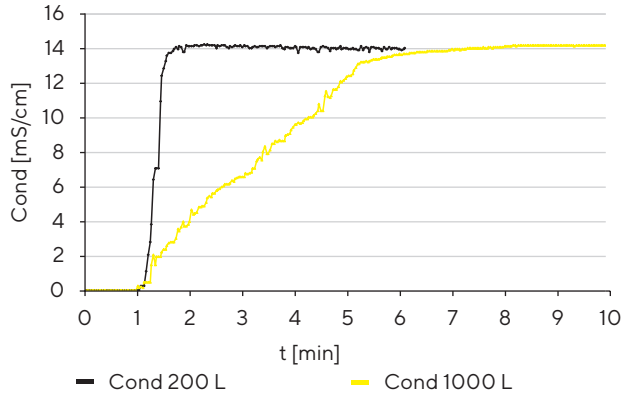


Figure 11:
Media powder dissolution – Comparison between 200 L and 1000 L scales

2. Mixing Performances vs Media Type

The mixing time reported in the table represents only the time to dissolve the media powders at the different scales. These mixing times include the transfer time of the multiple Sartorius Stedim Biotech Powder Transfer Bags System (for the 200 L scale) or SAFC® bucket liners (50 and 1000 L scale experiments).

Volume [L]	Control test	50	200	1000
Media powder				
Ex-Cell™ CD CHO Fusion	Conductivity Mixing time 1		< 1 min	< 5 min
	Visual inspection Mixing time 2	< 2 min	< 4 min	< 10 min
Ex-Cell™ EBx® GRO-I	Conductivity Mixing time 1		< 1 min	< 5 min
	Visual inspection Mixing time 2	< 4 min	< 4 min	< 15 min

Figure 12: Overview on mixing times regarding media powder dissolution

Note: conductivity value not stable at 50 L due to the presence of air bubbles around the conductivity cell (strong vortex)

The process time for both media preparation including water filling, media powder addition, sodium bicarbonate addition, acid and base adjustment if needed and final dilution take around 15 minutes for 50 L and 200 L scale and around 45 minutes for 1000 L scale.

Conclusions

- Large volume media solutions are quick and easy to prepare using the combination of ready to use media formulations and the high efficiency mixing of the Flexel® with Magnetic Mixer Technology.
- The contained processing conditions with the closed Powder Transfer Bag System docked onto the sterile Flexel® Bag for Magnetic Mixer are favourable to maintain low bioburden and to reduce to the minimum exposure of the operator to chemicals.
- The platform provides a single-use scalable media preparation capability with a range of Flexel Bags including volumes of 50 L, 100 L, 200 L, 400 L, 650 L and 1000 L.
- Flexel® for Magnetic Mixing system will be integrated in a Flexact® MP system and will provide monitoring and automation capability to better control all the operations of media preparation step.

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