SARTORIUS

Application Note

May 2025

Keywords or phrases:
Biostat® RM, rocking motion bioreactor, single-use bioreactor, mRNA, in vitro transcription (IVT), batch, fed-batch, wave, PATfix® mRNA platform

mRNA Production in GMP-Compliant Biostat® RM Systems

Bernward Husemann[1], Janja Skok[2], Polona Megušar[2], Tina Vodopivec Seravalli[2], Jens-Christoph Matuszczyk[3], Tobias Schenk[3], Rok Sekirnik[2]
[1] Sartorius Stedim Systems GmbH, Robert-Bosch-Straße 5 – 7, 34302 Guxhagen, Germany
[2] Sartorius BIA Separations d.o.o., Mirce 21, 5270 Ajdovščina, Slovenia
[3] Sartorius Stedim Biotech GmbH, August-Spindler-Strasse 11, 37079 Goettingen, Germany

Correspondence
Email: bernward.husemann@sartorius.com

Abstract

The growing demand for RNA-based therapies requires solutions for GMP in vitro transcription (IVT) at small and mid-scale.

This application note shows the simple and successful transfer of a batch IVT process from a thermal shaker to the Biostat® RM. Comparable final mRNA concentrations were achieved when the volume was increased from 100 µL to 100 mL, showing that scale-up between both technology platforms at 1000-fold different volume scales is feasible. By using a fed-batch IVT approach, mRNA production was increased, and the final mRNA yield of 2 g was more than double the yield obtained during the batch IVT.

Introduction

RNA-based therapies, such as those relying on mRNA, saRNA, and circRNA, are gaining popularity due to their potential to treat various diseases. During the pandemic, larger volumes of these products were required for global-scale vaccine production. Now, production needs are shifting to smaller patient populations based on indications like influenza or cancers. To address this issue, we aimed to establish a proof-of-concept for GMP production of mRNA using in vitro transcription (IVT) at 100 mL scale with the Biostat® RM, a single-use bioreactor. This bioreactor can serve as a tool for small-volume processes while also providing a framework for seamless scale-up to larger scales using the Biostat® platform. Traditionally, the IVT reaction is performed as a batch reaction. Recently, a successful fed-batch IVT approach using the Ambr® 250 Modular bioreactor system was reported.[1] We adapted the protocols for both batch and fed-batch processes to the Biostat® RM and compared the results to those obtained with a thermal shaker and the Ambr® 250 Modular. The protocol also incorporated at-line measurement of mRNA production and material consumption using PATfix®.

Materials and Methods

Equipment

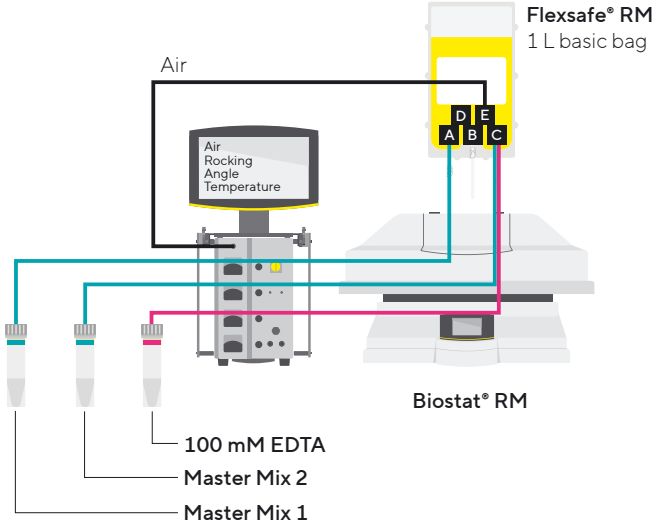
- Biostat® RM 20 | 50 bioreactor system with 20 L tray
- Sterile Flexsafe® RM 1 L basic bag (DBF001L)
- Analytical system (PATfix®)
- Pump tubing with inner diameter of 0.8 mm
- Pump tubing with inner diameter of 3.1 mm
- Sterile 50 mL feed container
- Sterile 100 mL feed container
- 1.5 mL tube
- Sterile 1 mL syringe
- Sterile 50 mL syringe
- Sterile needle

System Setup

A standard Biostat® RM 20 | 50 system was used for this study. The control tower has several internal pumps for precise liquid addition, and the rocker was equipped with a 20 L tray. A sterile standard Flexsafe® RM 1 L bag with five ports was used as a reaction vessel.

The pump tubing with an inner diameter of 0.8 mm was cut to the required length to connect to port A of the Flexsafe® RM bag on one side, be inserted into pump A, and reach the master mix 1 feed container on the other (Figure 1).

Figure 1: *Experimental Setup of the System for a Batch In Vitro Transcription Experiment*



The same tubing length was used to connect the master mix 2 feed container via pump B with port C of the Flexsafe® RM bag. The pump tubing with an inner diameter of 3.1 mm was cut to the required length to reach from the Flexsafe® RM bag on the one side, be inserted into pump C, and reach the EDTA feed container on the other.

Preparation of IVT Reagents for the Batch Reaction

1. All IVT reagents (except enzymes) were thawed and pre-heated at 37 °C.
2. Enzymes were equilibrated at room temperature.
3. For the 100 mL batch IVT reaction, two master mixes were prepared: one containing all the enzymes and the other containing all the other IVT reagents (Tables 1 and 2).
4. Master mixes were mixed individually with sterile pipettes. Nuclease-free water was divided between both master mixes to fill the total volume up to 50 mL.

Table 1: *Master Mix 1 for the Batch In Vitro Transcription Reaction*

Reagent	Bulk Concentration	Concentration in Final Volume (100 mL)	Volume [mL]
Nuclease-free water	—	—	6.00
10 × IVT buffer	10.00	1.00	10.00
ATP	100.00 mM	8.00 mM	8.00
GTP	100.00 mM	8.00 mM	8.00
CTP	100.00 mM	8.00 mM	8.00
UTP	100.00 mM	8.00 mM	8.00
Linearized pDNA	1.00 mg/mL	0.02 mg/mL	2.00
Total volume	—	—	50.00

Note. 10 × IVT buffer (400 mM Tris, 300 mM $MgCl_2$, 10 mM DTT, 20 mM Spermidine, pH 7.9 at 25 °C) was prepared in-house.

Table 2: *Master Mix 2 for Batch In Vitro Transcription Reaction*

Reagent	Bulk Concentration	Concentration in Final Volume (100 mL)	Volume [mL]
Nuclease-free water	—	—	26.5
RNase inhibitor	40.0 U/µL	1.0 U/µL	2.5
Pyrophosphatase	1.0 mg/mL	1.0 U/µL	1.0
T7 RNA polymerase	20.0 mg/mL	10.0 U/µL	20.0
Total volume	—	—	50.0

Note. For quenching, 43 mL of EDTA 100 mM pH 8.0 was used.

Preparation of IVT Reagents for Fed-Batch Reaction

The fed-batch protocol was adapted from Skok et al. (2022).[1]

1. All IVT reagents (except enzymes) were thawed and pre-heated at 37 °C.
2. Enzymes were equilibrated at room temperature.
3. For the 100 mL fed-batch IVT reaction, two master mixes were prepared: one containing all the enzymes and the other containing all the other IVT reagents (Tables 3 and 4).
4. Master mixes were mixed individually with sterile pipettes. Nuclease-free water was divided between both master mixes to fill the total volume up to 50 mL.
5. The feed for the fed-batch IVT was a mixture of NTPs and $MgCl_2$ (Table 5) mixed in a 100 mL sterile feed container.

Table 3: *Master Mix 1 for Fed-Batch In Vitro Transcription Reaction*

Reagent	Bulk Concentration	Concentration in Final Volume (100 mL)	Volume [mL]
Nuclease-free water	—	—	3.00
10 × IVT buffer	10.00	1.00	10.00
ATP	100.00 mM	8.00 mM	8.00
GTP	100.00 mM	8.00 mM	8.00
CTP	100.00 mM	8.00 mM	8.00
UTP	100.00 mM	8.00 mM	8.00
Linearized pDNA	1.00 mg/mL	0.02 mg/mL	5.00
Total volume	—	—	50.00

Note. 10 × IVT buffer (400 mM Tris, 300 mM $MgCl_2$, 10 mM DTT, 20 mM Spermidine, pH 7.9 at 25 °C) was prepared in-house.

Table 4: *Master Mix 2 for Fed-Batch In Vitro Transcription Reaction*

Reagent	Bulk Concentration	Concentration in Final Volume (100 mL)	Volume [mL]
Nuclease-free water	—	—	26.5
RNase inhibitor	40.0 U/µL	1.0 U/µL	2.5
Pyrophosphatase	1.0 mg/mL	1.0 U/µL	1.0
T7 RNA polymerase	20.0 mg/mL	10.0 U/µL	20.0
Total volume	—	—	50.0

Table 5: *Feed for Fed-Batch In Vitro Transcription Reaction*

Reagent	Bulk Concentration	Concentration in Final Volume (100 mL)	Volume [mL]
Nuclease-free water	—	—	0.48
ATP	100.00 mM/L	8.00 mM	18.50
GTP	100.00 mM/L	8.00 mM	18.50
CTP	100.00 mM/L	8.00 mM	18.50
UTP	100.00 mM/L	8.00 mM	18.50
1 M $MgCl_2$	—	—	5.52
Total volume	—	—	80.00

Note. For quenching, 344 mL of EDTA 100 mM pH 8.0 was used.

Pump Speed Determination

A correlation between the pump speed setpoint [%] and the flow rate was determined before the IVT experiments. This established proper alignment, especially for the low pump speed setpoints between 5 and 20% needed for the fed-batch IVT experiment.

At each defined setpoint, water or a liquid of similar viscosity to the IVT solution was pumped for at least 10 minutes. The flow rate for each setpoint was calculated by measuring the weight of the pumped liquid.

Mixing Test

The quality of mixing of different solutions is an important parameter for the IVT reaction. Therefore, a mixing test was performed, mimicking an IVT reaction. The system was set up as described in the "System Setup" chapter. For master mix 1, 50 mL of water was used, and for master mix 2, a mixture of 1:1 water with dye and glycerol was used to mimic the viscosity of the enzyme stock. The bag was inflated and then aerated with a constant airflow of 0.1 lpm to keep the Flexsafe® RM bag properly bloated. The rocking speed was set to 20 r/min at an angle of 7°. Then, pump A and pump B were started. All parameters were the same as for the IVT experiments. The observed mixing of the colored liquids showed sufficient mixing for the IVT reaction. Therefore, the defined rocking speed of 20 r/min at an angle of 7° was used for the IVT experiments.

Batch IVT in the Biostat® RM

Experimental Preparation

After sanitization, the master mixes were prepared, and the Biostat® RM system was set up. The Flexsafe® RM bag was placed on the rocker. The tubing for master mix 1 was connected to the Flexsafe® RM bag at port A and put in pump A. The tubing for master mix 2 was connected to the Flexsafe® RM bag at port C and put in pump B. The tubing for EDTA addition was put in pump C but not connected to the Flexsafe® RM bag. For gassing, tubing was connected at port E of the Flexsafe® RM bag and the air outlet of the control tower. The bag was then covered with the lid. The control tower was connected to BioPAT® MFCS for recording data and supervising parameters.

The bag was inflated with air at 1 lpm. After inflation, the airflow was reduced to 0.1 lpm and maintained throughout the experiment to keep the bag bloated. The rocking was started after setting the rocking speed to 20 r/min and the angle to 7°. The temperature control was then started by setting a temperature setpoint of 37 °C.

The feed containers with the master mixes and the EDTA were connected to the respective tubing and put on the scale (Figure 2).

Figure 2: *Experimental Setup of the Biostat® RM System for the Batch IVT Experiment*

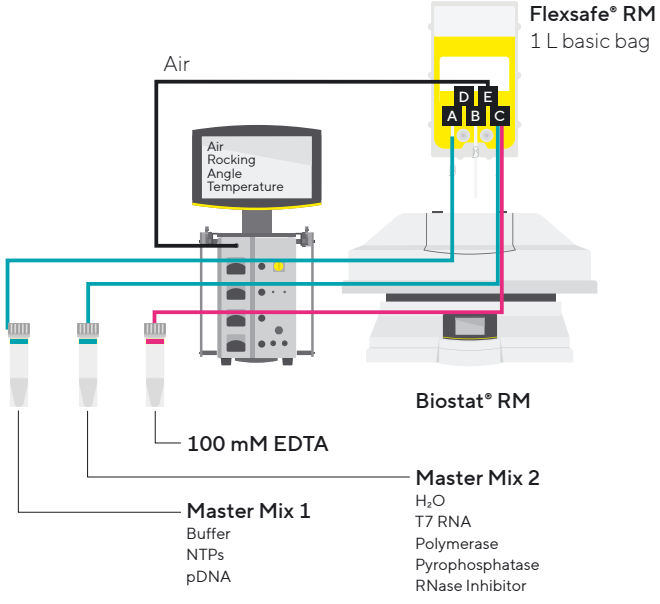
Port A
Master mix 1 at 5.6 mL/min

Port B
Sampling with 1 mL syringe

Port C
Master mix 2 at 5.7 mL/min or inactivation solution (100 mL EDTA) at 20 mL/min

Port D
Air outlet with filter and filter heater

Port E
Air inlet with filter



Experimental Execution

Once the experimental setup was complete, both pump A and pump B were turned on with a pump speed setpoint of 100%. Due to slight differences in the pump-tubing combination, this resulted in a flow rate of 5.6 mL/min for pump A and 5.7 mL/min for pump B. After approximately 10 minutes, the feed containers of both master mixes were emptied, and both pumps were kept running until the tubing was fully cleared. This minimized the loss of master mix due to the tubing's dead volume.

After the complete addition of both master mixes, the first sample was taken with a sterile 1 mL syringe. The following sampling was performed every 30 minutes. During sampling, the rocker was set to the sampling mode. The sterile syringe was connected to port B of the Flexsafe® RM bag, and a 100–500 µL sample was taken. The sample was transferred to a 1.5 mL tube and quenched with 100 mM EDTA at a 1:1 ratio. The same syringe was used to blow air into port B, pushing the liquid back inside the sampling tubing to minimize volume loss. After the first sample was taken, the tubing of master mix 2 was disconnected from port C, and the EDTA tubing was connected to port C.

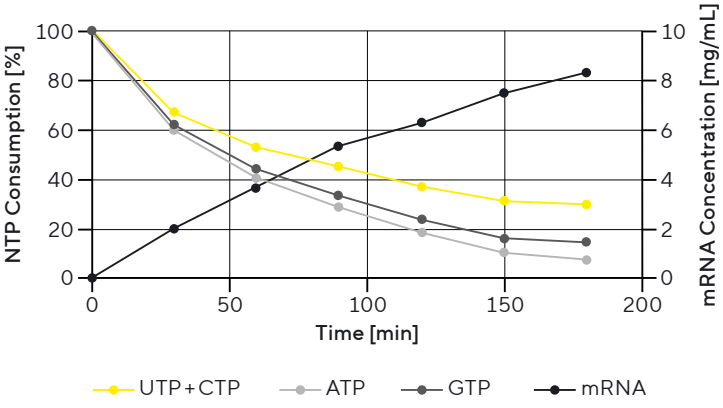
The samples were analyzed at-line by a CIMac PrimaS® column to monitor mRNA production and the NTP consumption during the experiment. When the plateau of mRNA production was reached, confirmed by the PATfix® mRNA Platform using PrimaS analytics, pump C was turned on to add EDTA to quench the reaction. The pump speed was set to 100%, resulting in a 20 mL/min flow rate. As done with the addition of the master mixes, the EDTA tubing was purged with air by keeping pump C running until the EDTA tubing was fully emptied. After pump C was turned off, the rocking continued for an additional 2 minutes, allowing thorough mixing of the reactants with EDTA and efficient quenching.

After the reaction was quenched, the Biostat® RM was stopped, and all ports were closed. The liquid was harvested from the bag through port B using a sterile 50 mL syringe. The quenched IVT was purified as described previously.[1]

Results and Discussion

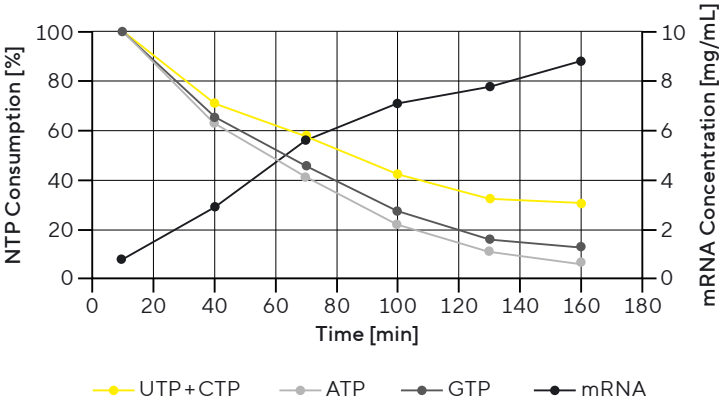
As a reference, a 100 µL IVT batch experiment was performed in a thermal shaker in a 1.5 mL tube (Figure 3). In this experiment, the NTP concentration gradually decreased, while the concentration of mRNA increased steadily until reaching 8.3 mg/mL after 180 minutes.

Figure 3: *Results of the Batch IVT Reference Experiment in a Thermal Shaker With 100 µL Liquid Volume*



The batch IVT experiment in the Biostat® RM with 100 mL liquid volume (Figure 4) shows NTP consumption and mRNA production comparable to the 100 µL experiment in the thermal shaker. The batch IVT reaction in the Biostat® RM was slightly faster than in the thermal shaker, reaching the final concentration after 160 minutes. The final NTP concentrations were almost the same between the two methods, as was the mRNA concentration (8.8 mg/mL for the 100 mL Biostat® RM experiment), yielding a total of 0.88 g mRNA.

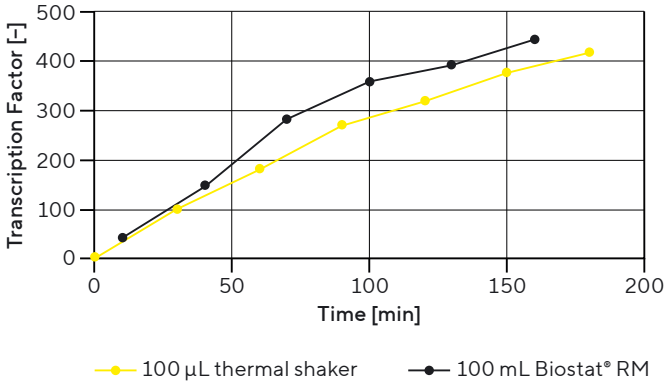
Figure 4: *Results of the Batch IVT Experiment in the Biostat® RM With 100 mL Liquid Volume*



The transcription factors (defined as mg mRNA/mg pDNA) for the batch IVT reaction in the thermal shaker and the Biostat® RM were similar.

They showed nearly linear trends throughout the experiments, with a slightly higher slope in the Biostat® RM (Figure 5). Therefore, the reaction kinetics in the Biostat® RM were comparable to the kinetics of the control reaction performed in the thermal shaker, showing that method transfer between both technology platforms at 1,000-fold different volume scales is feasible.

Figure 5: *Transcription Factors of the Batch IVT Experiments in the Thermal Shaker (100 µL) and the Biostat® RM (100 mL)*



Fed-Batch IVT in the Biostat® RM

Experimental Preparation

After sanitization, the master mixes and the feed were prepared, and the Biostat® RM system was set up. The Flexsafe® RM bag was placed on the rocker. The tubing for master mix 1 | feed was connected to the Flexsafe® RM bag at port A and put in pump A. The tubing for master mix 2 was connected to the Flexsafe® RM bag at port C and put in pump B. The tubing for EDTA addition was only put in pump C but not connected to the Flexsafe® RM bag. For gassing, tubing was connected at port E of the Flexsafe® RM bag and the air outlet of the control tower. The bag was then covered with the lid. The control tower was connected to BioPAT® MFCS for data recording and supervision of the parameters.

The bag was inflated with air at 1 lpm. After inflation, the airflow was reduced to 0.1 lpm and maintained throughout the experiment to keep the bag bloated. The rocking was started after setting the rocking speed to 20 r/min and the angle to 7°. The temperature control was then started by setting a temperature setpoint of 37 °C.

The containers holding the master mixes, the feed, and the EDTA were connected to the respective tubing and put on the scale (Figure 6).

Figure 6: *Experimental Setup of the Biostat® RM System for the Fed-Batch IVT Experiment*

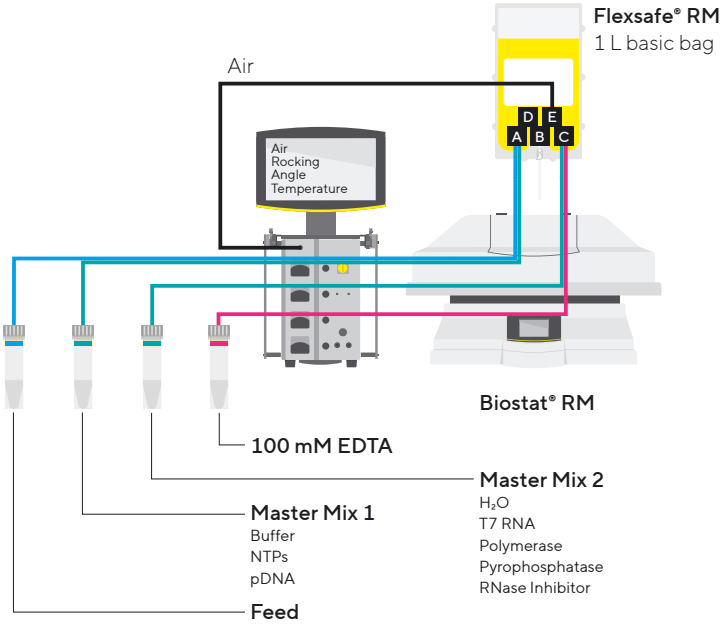
Port A
Master mix 1 at 5.6 mL/min

Port B
Sampling with 1 mL syringe

Port C
Master mix 2 at 5.7 mL/min or Inactivation solution (100 mL EDTA) at 20 mL/min

Port D
Air outlet with filter and filter heater

Port E
Air inlet with filter



Experimental Execution

Once the experimental setup was complete, both pump A and pump B were turned on with a pump speed setpoint of 100%. Due to slight differences in the pump-tubing combination, this resulted in a flow rate of 5.6 mL/min for pump A and 5.7 mL/min for pump B, respectively. After the feed containers of both master mixes were emptied, both pumps were kept running until the tubing was fully cleared. This minimized the loss of master mix due to the tubing´s dead volume.

After the complete addition of both master mixes, the first sample was taken with a sterile 1 mL syringe. The following sampling was performed regularly. During sampling, the rocker was set to sampling mode. The sterile syringe was connected to port B of the Flexsafe® RM bag, and a 100 – 500 µL sample was taken.

The sample was transferred to a 1.5 mL tube and quenched with 100 mM EDTA at a 1:1 ratio. The same syringe was used to blow air into port B, pushing the liquid back inside the sampling tubing to minimize volume loss. After the first sample was taken, the tubing of master mix 2 was disconnected from port C, and the EDTA tubing was connected to port C.

After 30 minutes of incubation, the feeding was initiated. The tubing of master mix A was disconnected, the tubing connected to the feed, and pump A started. The feeding regime as described in Skok et al (2022)[1] was adapted for continuous feeding with the Biostat® RM.

The samples were analyzed at-line by a CIMac PrimaS® column to monitor mRNA production and NTP consumption during the experiment. The feed flow rates were adapted depending on the analytical results. After the final volume was reached, the feeding was stopped. Pump C was turned on to quench the reaction with EDTA. The pump speed was set to 100%. As done with the addition of the master mixes, the EDTA tubing was purged with air by keeping the pump C running until the EDTA tubing was completely emptied. Once the EDTA tubing was completely emptied, the rocking continued for an additional 2 minutes to ensure the IVT liquid was thoroughly mixed with EDTA and the IVT reaction was quenched.

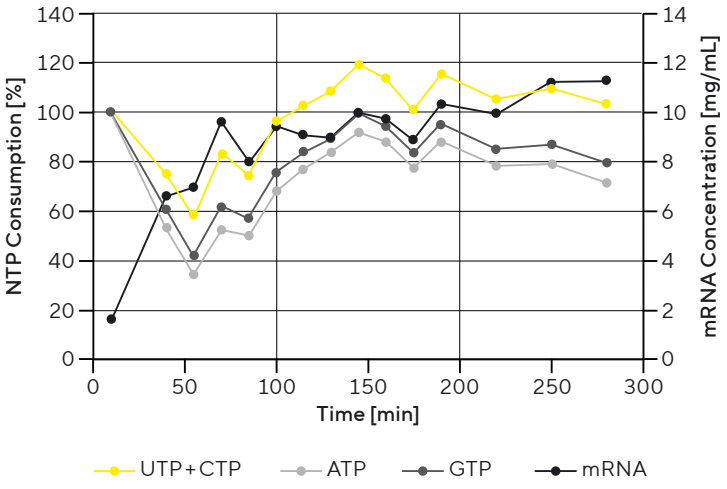
After the reaction was quenched, the Biostat® RM was stopped, and all ports were closed. The liquid was harvested from the bag through port B using a sterile 50 mL syringe. The quenched IVT was purified as described previously.[1]

Results

The first phase of the fed-batch experiment was a batch IVT reaction. A steep decrease in NTP concentration was observed (Figure 7). After the start of feeding, the NTP concentration increased again. The NTP+Mg feed was added at different flow rates to add NTP and Mg at the same rate as mRNA was produced and NTPs were consumed. After the feeding was stopped, the NTP concentration started to decrease again.

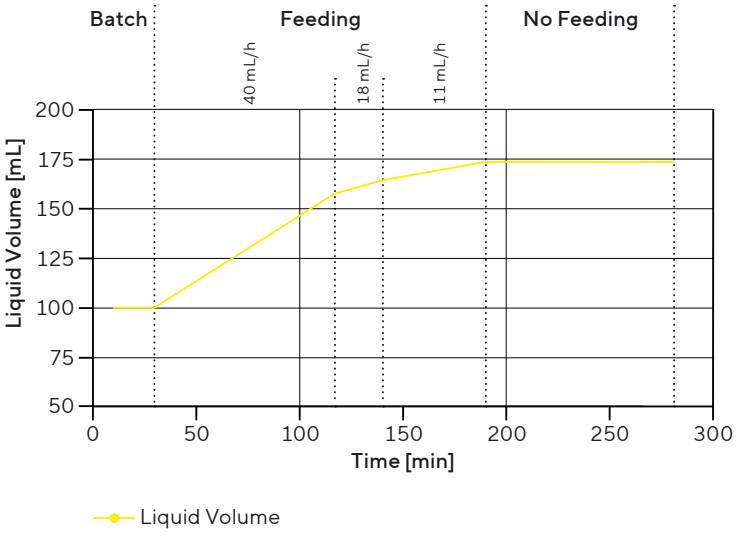
In the batch phase, a steep increase in the mRNA concentration was observed. In the feeding phase, the increase was lower than in the batch phase due to continuous dilution with the NTP+Mg feed.

Figure 7: *Results of the Fed-Batch IVT Experiment in the Biostat® RM*



The liquid volume of the fed-batch IVT experiment was calculated based on the pump output (Figure 8). Like in the fed-batch IVT experiments performed in the Ambr® 250, the feeding started at 40 mL/h and was reduced over the duration of the experiment to avoid overfeeding. The feeding reduction was calculated based on the results of the PATfix® analytical system.

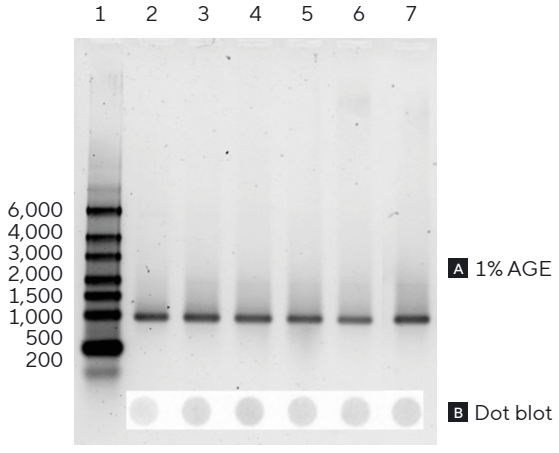
Figure 8: *Liquid Volume of the Fed-Batch IVT Experiment in the Biostat® RM, With Feed Phases Were as Follows: 30–117 min at 40 mL/h, 117–140 min at 18 mL/h, and 140–190 min at 11 mL/h*



The experiment was stopped after 280 minutes once a total volume of 174 mL was reached, with a final mRNA concentration of 11.3 mg/mL. This was equal to a production of 1.97 g of mRNA and comparable to the total mRNA reached in fed-batch IVT experiments in the Ambr® 250. Additionally, the total mRNA production increased by more than 2-fold compared to mRNA produced in the batch IVT performed in the Biostat® RM. The fed-batch IVT reaction in the Biostat® RM could theoretically continue until the maximum vessel volume of 500 mL is reached. Assuming the kinetics of mRNA production remained essentially constant, more than 5.6 g of mRNA could be produced in a single fed-batch IVT run. Based on a standard IVT productivity of 5 g/L2, in a batch process, this quantity of mRNA would require a vessel with a liquid volume of more than 1 L.

mRNA integrity was checked by agarose gel electrophoresis, and dsRNA presence was assessed using a dot blot. No visible mRNA degradation or increase in dsRNA was observed throughout the experiment, showing that a fed-batch IVT process in the Biostat® RM is suitable for mRNA production (Figure 9).

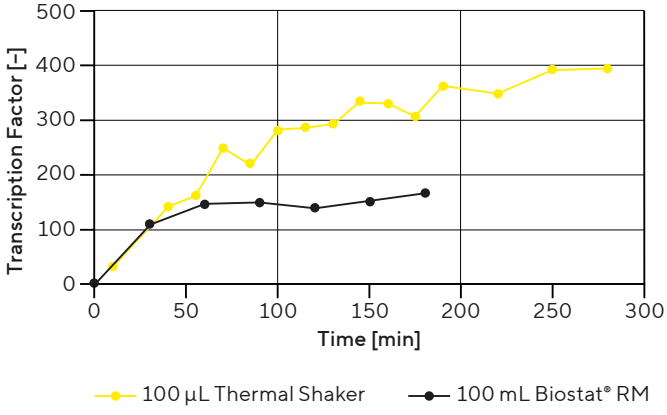
Figure 9: ***A)*** *1% Agarose Gel Electrophoresis (AGE) of Samples Throughout the Biostat® RM Fed-Batch Reaction and* ***B)*** *dsRNA*



Note. 1 – Ladder (RiboRuler), 2 – IVT 30 min, 3 – IVT 60 min, 4 – IVT 120 min, 5 – IVT 180 min, 6 – IVT 240 min, 7 – IVT inactivated

Initially, during the batch IVT phase, the transcription factor trends for the thermal shaker and the Biostat® RM were comparable. Then, the transcription factor remained constant for the IVT batch in the thermal shaker as the reaction reached a plateau due to the NTPs being fully consumed. For the fed-batch IVT experiment in the Biostat® RM, the production of mRNA continued after the feeding was started. Therefore, an approximately linear increase throughout the complete feeding phase was observed (Figure 10).

Figure 10: *Transcription Factors of the Fed-Batch IVT Experiment in the Biostat® RM and the Corresponding Reference Batch IVT Experiment in the Thermal Shaker*



Conclusion

The Biostat® RM is a well-established single-use bioreactor with bag sizes suitable for liquid volumes ranging from 100 mL to 100 L and is powered by Biobrain®, a fully GMP-compliant automation platform. The objective of these experiments was to demonstrate the direct transfer and easy scale-up of a batch IVT and a fed-batch IVT process from a thermal shaker at 100 µL to a Biostat® RM at 100 mL.

In the batch IVT, comparable final mRNA concentrations of around 8.5 mg/mL were reached in both processes. When using the fed-batch IVT process in the Biostat® RM, a final mRNA concentration of 11.3 mg/mL was reached, resulting in a final mRNA yield of 2 g. These concentrations and yields were comparable to the results achieved in the Ambr® 250.

These results show that the standard Biostat® RM is a suitable technology platform to perform multi-gram mRNA synthesis, matching the capabilities of a stirred bioreactor system. The GMP-ready bioreactor has multiple advantages over traditional thermal shakers, such as robust inline monitoring and control of pH and temperature in real-time and precisely controlled and automated feed additions. This supports further optimization of feed addition and improved manufacturing conditions. Implementing recipes for automated feed strategies can reduce manual handling and improve the reproducibility of the results. Due to the use of single-use materials, the sterile bags, tubing, and feed containers can be discarded after each run, requiring no or minimal cleaning between the production runs.

Automated single-use bioreactor systems — especially in fed-batch mode — can facilitate continuous mRNA manufacturing by significantly decreasing manual handling and the cost of goods. As a result, they contribute to lowering the cost of mRNA production by simplifying and intensifying manufacturing.

References

1. Skok, J, Megusar, P., et al. Gram-scale mRNA Production Using a 250 mL Single-Use Bioreactor, Chemie Ingenieur Technik, 2022, 94, No.12, 1928 – 1935.

2. J. M. Henderson, A. Ujita, E. Hill, S. Yousif-Rosales, C. Smith, N. Ko, T. McReynolds, C. R. Cabral, J. R. Escamilla-Powers, M. E. Houston, Curr. Protoc. 2021, 1 (2), e39. DOI: https:// doi.org/10.1002/cpz1.39

Germany
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Stedim North America Inc.
565 Johnson Avenue
Bohemia, NY 11716
Toll-Free +1 800 368 7178

For more information, visit
sartorius.com

Last modified: 11 | 2025