


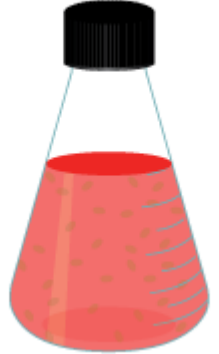



# Liquid Injection System (LIS)

Automated Feeding of Liquids in Shake Flasks

Currently, researchers can perform automatic feeding experiments in both bioreactors and microtiter plates, but not in shake flasks, which is limiting their use for bioprocess development.

Problem: Lack of feeding technologies for shake flask cultures

|                                | Microtiter Plate  | Shake Flask  | Bioreactor   |
|--------------------------------|---|--|--|
| Feeding technologies available | <br>✓ | <br>✗ | <br>✓ |
| Feeding Applications           | Fed-batch, pH control, automated induction  | ✗  | Fed-batch, pH control, addition of antifoam, automated induction                         |

LIS is the first technology allowing for automated feeding of liquids into microbial shake flask cultures.

## LIS Technology

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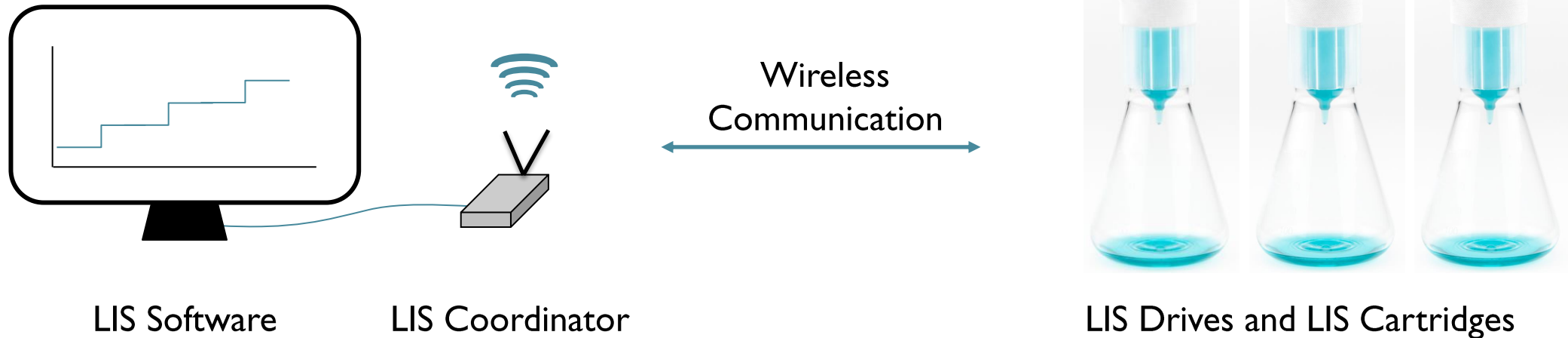


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The LIS Technology consists of 4 main components: the LIS Drive, LIS Cartridge, LIS Software and LIS Coordinator.

## Overview LIS Technology and its components

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The LIS Drive and the LIS Cartridge are the two key components of the LIS technology.

## LIS Components (1/2): LIS Drive and LIS Cartridge



The diagram illustrates the LIS Drive and LIS Cartridge components. The LIS Drive is a small black cylindrical device with a white base, shown in a callout box on the left. The LIS Cartridge is a clear plastic container with a blue liquid inside, shown in a callout box on the right. The main image shows the LIS Drive mounted on top of the LIS Cartridge, which is placed on a clear glass flask. The LIS Drive is connected to the LIS Cartridge via a white ring. The LIS Drive has a small display screen showing the following text:

```
Setup Run
Elapsed Time 00:00:00.00
Reservoir Time 00:00:00.00
Dispensed Volume 00.00 ml
Remaining Volume 25.00 ml
Back
```

### LIS Drive



- Programmable miniature pump
- Controls feeding of liquid from the LIS cartridge into the flask
- Is programmed wirelessly with the LIS Software
- Plug-and-Play

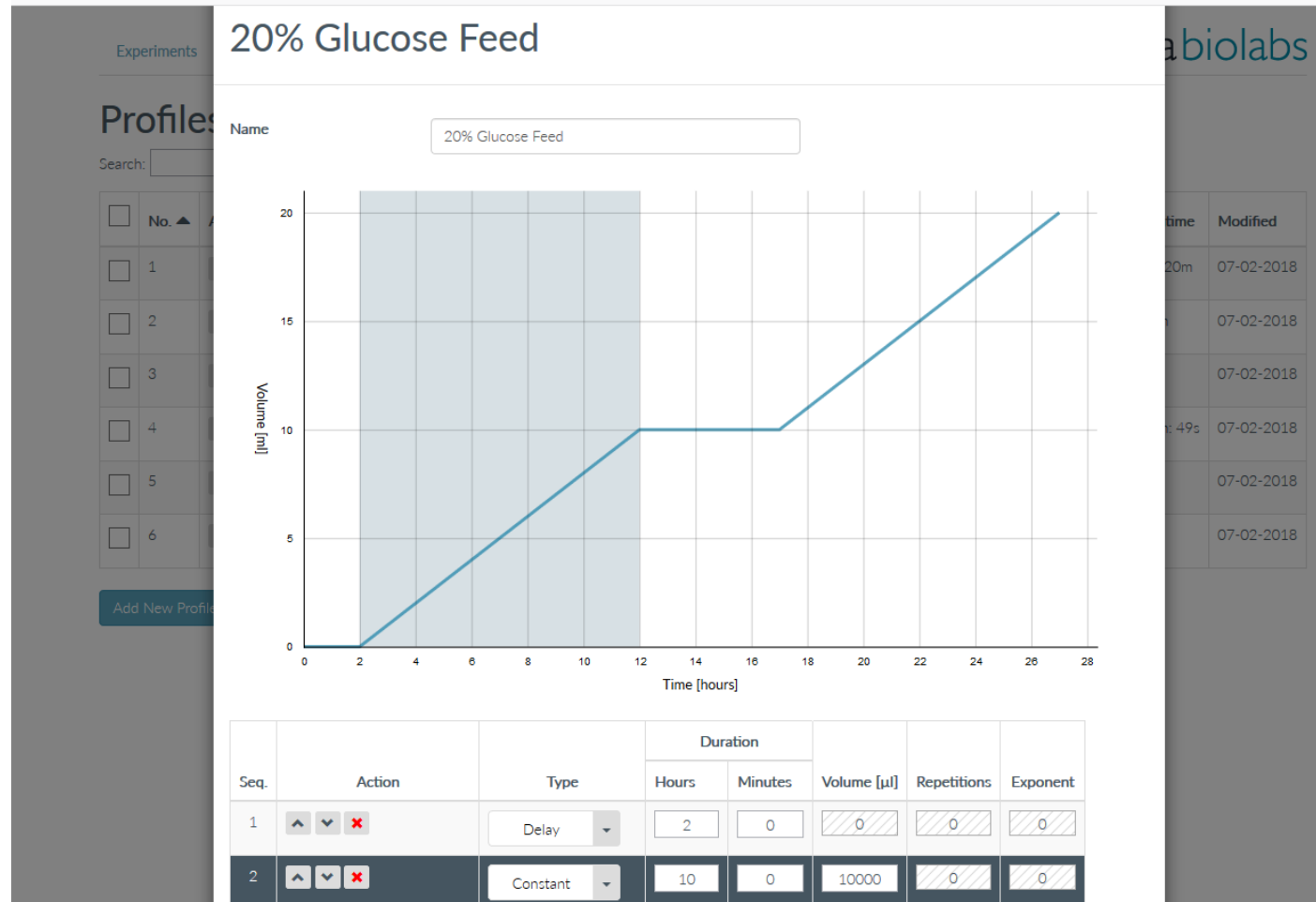
### LIS Cartridge



- Sterile container with the shape of a shake flask lid.
- Can be filled with up to 25ml of the desired feeding liquid
- Only compatible with 38mm straight neck flasks
- Single Use Consumable

The LIS Software together with the LIS Coordinator enables the user to set-up, control and monitor the LIS experiments wirelessly.

## LIS Components (2/2): LIS Software and LIS Coordinator



Researchers around the globe use the LIS technology for a broad variety of scientific applications in the area of bioprocessing.

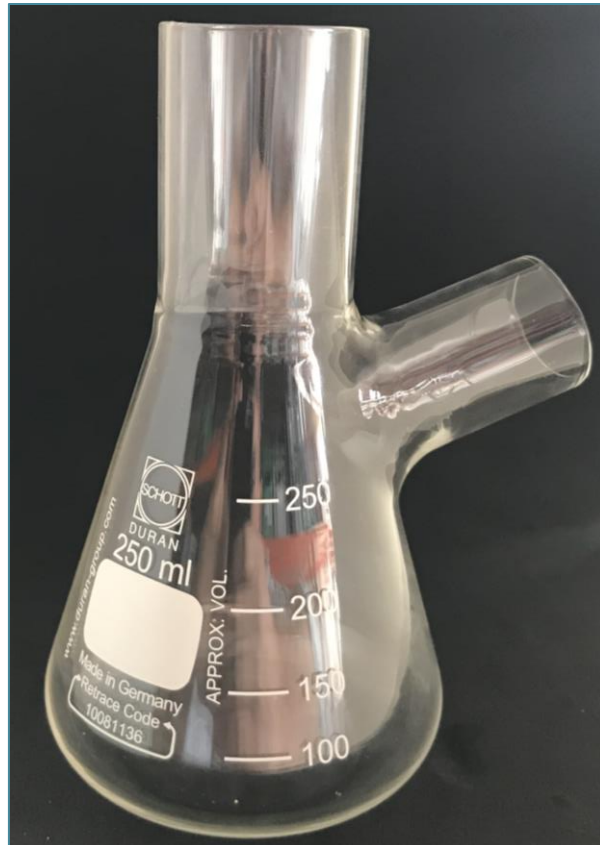
### Exemplary Applications for the LIS Technology

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- **Generation of Fed Batch conditions in shake flasks**
  - Align screening (shake flask) and production conditions (bioreactor)
  - Generate more biomass in shake flasks
  - Generate higher product titers in shake flasks
- **Automatic induction of protein expression**
  - To harvest protein in the morning for down-stream experiments, cultures would need to be induced at night when nobody is at work
  - With LIS one can induce cultures at night and thus optimize the timing of experiments, which creates efficiency and productivity gains for the lab
- **Phage screening**
- **Create a fresh pre-culture**
  - Automatically inoculate media overnight and harvest a fresh culture in the morning
- **pH control**
  - Stabilize pH by constantly adding acid/base
- ...

Optionally, customers can use our special LIS flasks instead of their standard shake flasks to meet the increased oxygen demand of their microbial cultures when performing experiments with LIS.

## LIS Flasks



### What are LIS Flasks?

During Fed-Batch experiments with LIS, many microbial bioprocesses show an increased demand for oxygen. LIS shake flasks have additional necks allowing you to meet the increased oxygen demand of your cultures.

### Specifications

- For microbial cultures
- Standard 38mm top neck for mounting of LIS
- 1 additional side neck for maximal oxygen intake
- 250, 500, 1000 and 2000ml shake flasks, with and without baffles
- Autoclavable glass flasks

### Benefits

Use your standard seal (e.g. cotton plug), while applying the LIS technology and avoid an oxygen limitation for your culture.

LIS compatible with cell cultures (e.g. CHO cells) currently under development.



LIS flasks can be used with all commonly used shake flask seals to generate good oxygen transfer rates.

### Examples of LIS Flask Side Neck Sealing Options

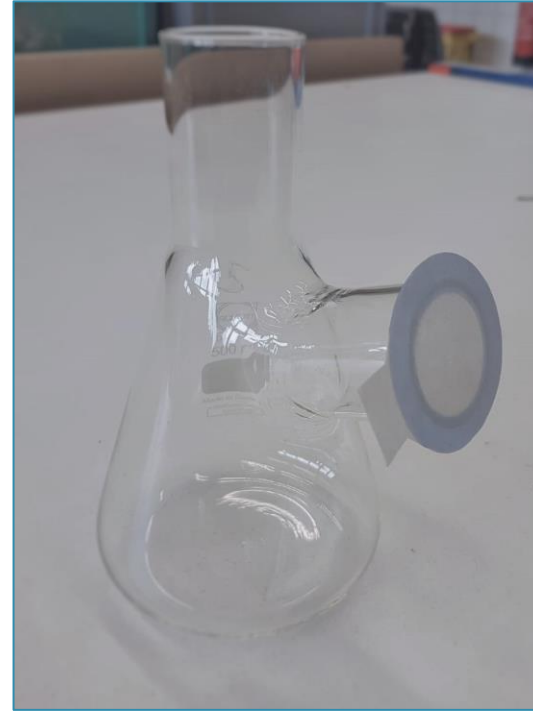
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Cotton plug



Aluminium cap



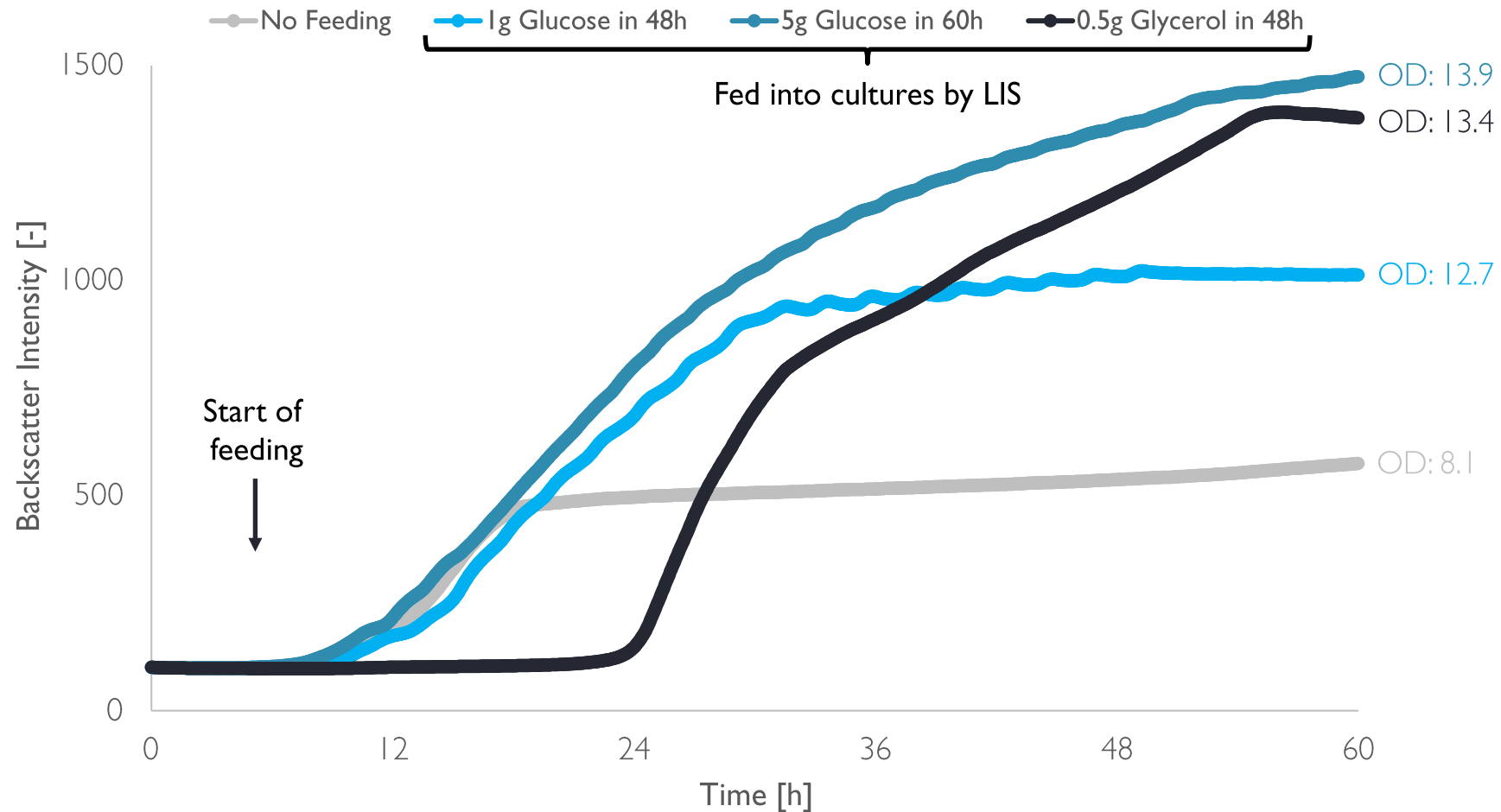
AirOtop seal



Silicone sponge closures

LIS can be used to create fed-batch conditions for microbial shake flask cultures and thus increase biomass and product titers.

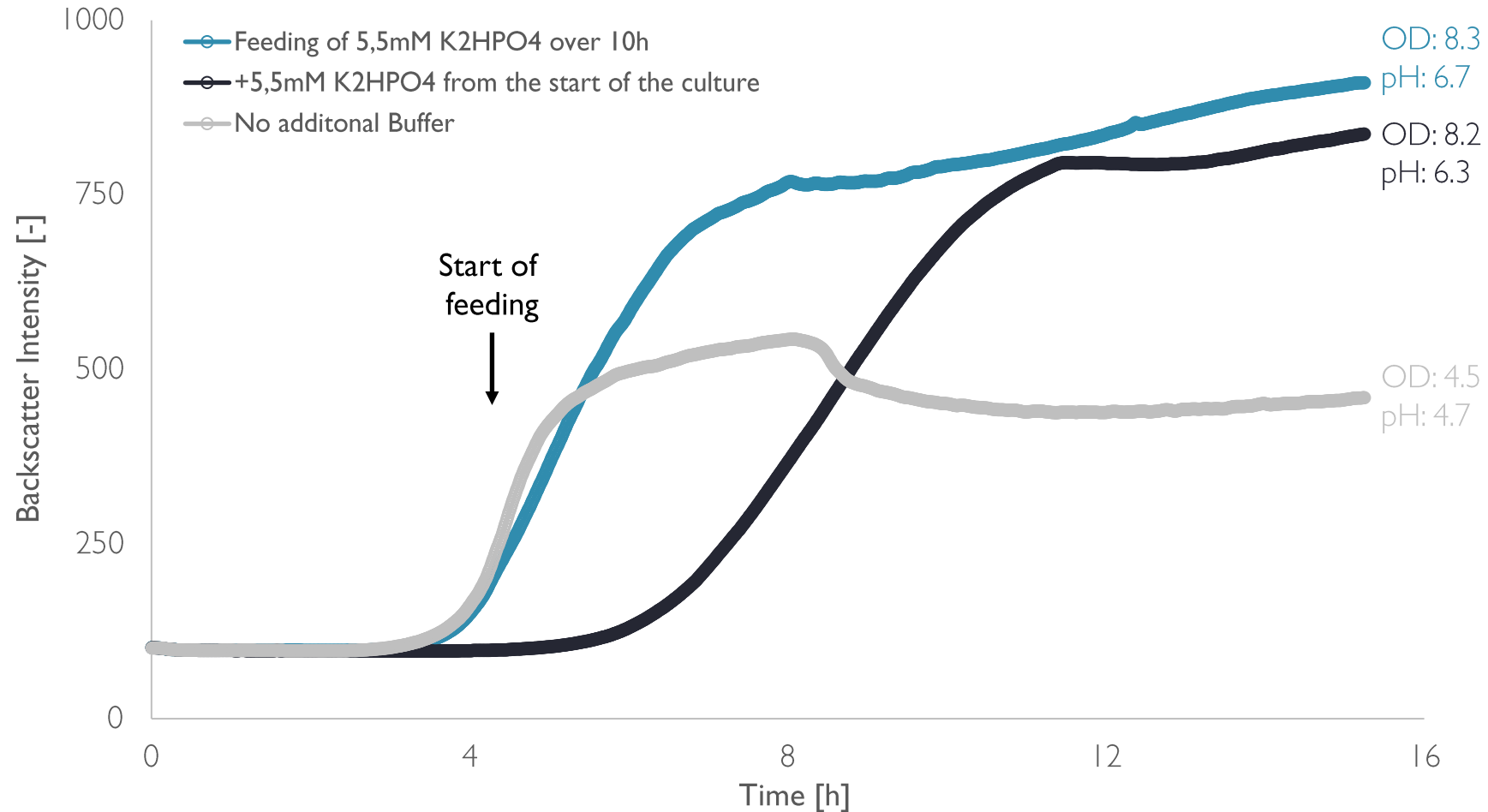
### Exemplary Use Case (1/3): Fed-Batch



*Saccharomyces cerevisiae*, 25 ml YPD Medium, 250 ml Shake Flasks, 30 °C, 250 rpm

LIS can be used to control pH drifts of *Escherichia coli* shake flask cultures by automatically feeding buffer to the culture.

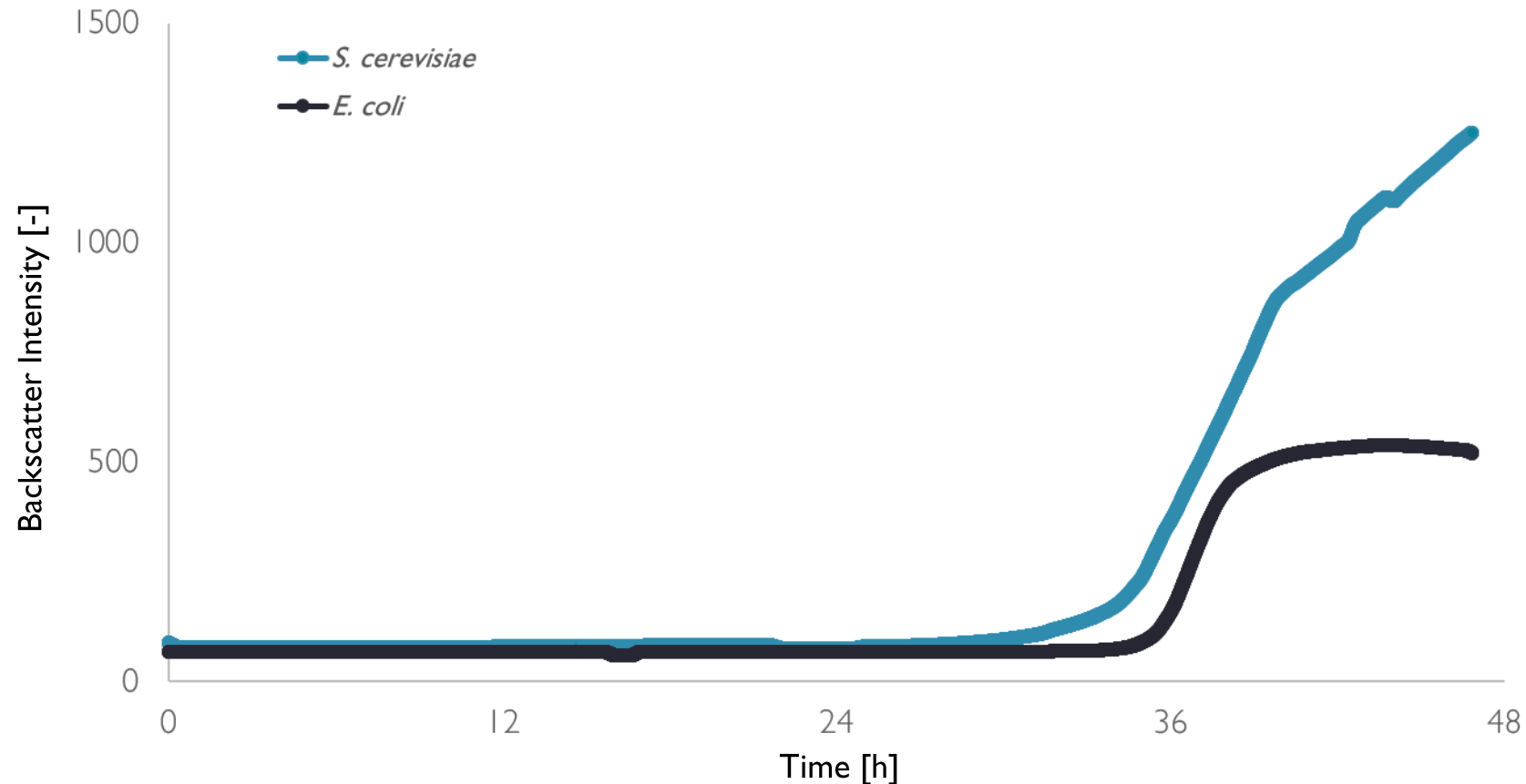
### Exemplary Use Case (2/3): pH Regulation



*Escherichia coli*, 25 ml LB Medium + 2 % Glucose, 250 ml Shake Flask, 37°C, 250 rpm

LIS can be used to time your precultures perfectly for the next experiment.

### Exemplary Use Case (3/3): Preculture Inoculation



The first LIS cartridge was filled with an *E. coli* culture (LB medium + Kanamycin, OD600 = 0.1). 1 ml of this culture was dispensed into a 250 ml shake flask filled with 25 ml LB + Kanamycin using a single shot after an initial delay of 32 h. The second cartridge was filled with a *S. cerevisiae* culture (YPD medium + 0.5 M NaCl, OD600 = 0.1). 1 ml of that culture was fed into a 250 ml shake flask filled with 25 ml YPD medium + 0.5 M NaCl using the same feeding profile.