

## Benchtop ELISA Automation with BenchCel Microplate Handler

### Introduction

The enzyme-linked immunosorbent assay (ELISA) is a common technique used in labs to detect a variety of biomolecules. The process of running ELISA plates requires several liquid handling steps between incubations and a final detection step. When performed manually, this is a laborious process that limits throughput and can introduce human error.

Automated systems increase throughput and assay precision, but some limit users to proprietary reagents and consumables, or are too large for benchtop implementation. The BenchCel Microplate Handler is a versatile benchtop system designed to move microplates between two instruments that are commonly used for ELISA; a microplate washer-dispenser and a microplate reader.



*Figure 1.* The BenchCel Microplate Handler, with integrated EL406 Washer Dispenser and Synergy Neo2 Multi-Mode Reader, fits easily on a standard lab benchtop.

Liquid handlers like BioTek's EL406™ automate the tedious steps required in a typical ELISA process. The EL406 is used for washing and ELISA reagent dispensing in a single platform. Multi-mode readers, like the Synergy™ Neo2, are used for the endpoint detection step of an ELISA, which is commonly done via absorbance (colorimetric) measurement. However, the Synergy Neo2 can also be used for fluorescence- and luminescence-based ELISA, offering assay flexibility over absorbance only readers.

Systems like these are well established in labs running ELISA, but a common bottleneck in this type of workflow is the manual intervention needed to move plates between the two instruments, limiting throughput and efficiency. The BenchCel microplate handler bridges the two instruments, enabling fully automated batch processing of ELISA plates post sample addition.

## Automating a Typical ELISA Workflow

When automating any multi-step laboratory protocol such as ELISA, throughput requirements must be balanced with assay requirements. Correct scheduling and batch size ensures that each sample is handled in the same fashion throughout, regardless of whether it is on the first plate or the last.

Important considerations for designing an automated workflow are:

- Identifying the rate limiting step
- Understanding what time constraints exist in the assay

Automated liquid handlers are typically faster than hand pipetting, but different operations within an ELISA workflow will have varying times. For instance, BioTek's EL406™ can add 100 µL of reagent to a 96-well microplate in 20 seconds, but a 5x wash cycle can take nearly a minute. An important time constraint in the ELISA workflow are the incubation periods following the additions of the secondary antibody, detection reagent, and stop reagent. The key is to ensure that intervals for processing the first plate at each sequential step is the same for processing the nth plate.

Consider the example ELISA protocol described below, run on the BenchCel Microplate Handler with the EL406 and Synergy™ Neo2. Each run begins after the sample has been added to the coated plate and the initial incubation has occurred. The subsequent steps and estimated timings are shown below. The timings of liquid handling and read steps are representative of those achievable on BioTek's EL406 and Synergy Neo2.

Assay Step	Volume	Duration
3x wash	350 µL	42 seconds
Dispense secondary antibody	100 µL	19 seconds
Incubation	NA	60 minutes
3x wash	350 µL	42 seconds
Dispense detection reagent	100 µL	19 seconds
Incubation	100 µL	10 minutes
Dispense stop reagent	100 µL	19 seconds
Absorbance read	NA	27 seconds

*Table 1.* Representative ELISA protocol schedule using BenchCel Microplate Handler, EL406 and Synergy Neo2.

Following this protocol, it would be possible to handle batch sizes of 9 plates while adhering to the incubation times set in the protocol, with the protocol requiring a total runtime of 81 minutes. In this case, it is assumed that each plate contains 80 samples while allotting the remaining wells for controls. In a 12-hour period, it would be feasible to run eight batches of this protocol, translating to a throughput of 72 plates or 5,760 samples. It is important to note that the throughput is determined by the timing constraints of the assay protocol, and not the processing capability of the automated system itself.

Protocol run time	81 minutes
# of 96-well plates per run	9
Average # of samples per plate	80
# of samples per run	720
Operation time per day	12 hrs
# of 96-well plates per 12 hr shift	72
<b>Total # of samples per 12-hr shift</b>	<b>5,760</b>

Table 2. Summary of representative ELISA throughput.

The VWorks dynamic scheduling software that underpins the operation of the system can be used to determine procedure runtimes through the use of embedded simulation modeling. The model can be updated to include timings for the individual protocol steps, to provide more accurate predictions. Before finalizing the automation procedure, it is best practice to perform a physical dry-run on the system to obtain real-world timings.

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