Technical Overview

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Benchtop ELISA Automation with an Agilent 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 Agilent 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.

Liquid handlers such as the Agilent BioTek EL406 washer dispenser automate the tedious steps required in a typical ELISA process. The EL406 is used for washing and ELISA reagent dispensing in a single platform. Multimode readers, such as the Agilent BioTek Synergy Neo2 hybrid multimode reader, are used for the endpoint detection step of an ELISA, which is commonly done through 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 such as 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 multistep 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 throughout fashion, 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, the 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 is 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 last plate.

Consider the example ELISA protocol described in Table 1, 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 listed. The timings of liquid handling and read steps are representative of those achievable on an EL406 and Synergy Neo2.

Following this protocol, it would be possible to handle batch sizes of nine plates while adhering to the incubation times set in the protocol, with the protocol requiring a total run time 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 (Table 2). The throughput is determined by the timing constraints of the assay protocol, and not the processing capability of the automated system itself.

The Agilent VWorks dynamic scheduling software that underpins the operation of the system can be used to determine procedure run times 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.

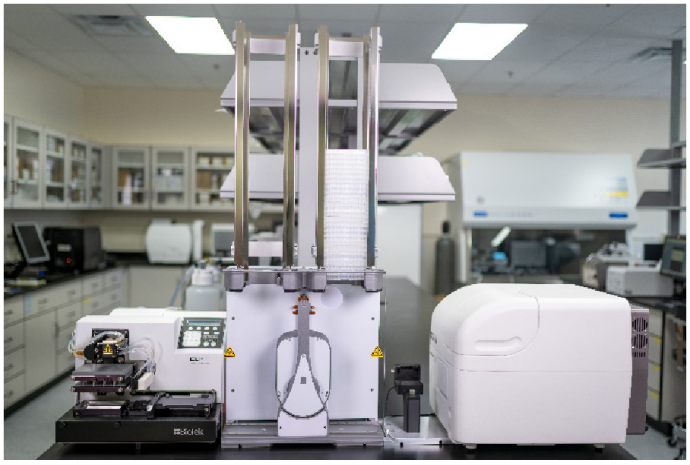

Figure 1. An Agilent BenchCel microplate handler with an integrated Agilent BioTek EL406 washer dispenser and Agilent BioTek Synergy Neo2 hybrid multimode reader fits easily on a standard lab benchtop.

Table 1. Representative ELISA protocol schedule using an Agilent BenchCel microplate handler, Agilent BioTek EL406 washer dispenser, and Agilent BioTek Synergy Neo2 hybrid multimode reader.

Assay Step	Volume	Duration
3x Wash	350 µL	42 s
Dispense Secondary Antibody	100 µL	19 s
Incubate	NA	60 min
3x Wash	350 µL	42 s
Dispense Detection Reagent	100 µL	19 s
Incubate	100 µL	10 min
Dispense Stop Reagent	100 µL	19 s
Read Absorbance	NA	27 s

Table 2. Summary of representative ELISA throughput.

Parameter	Value
Protocol Run Time	81 min
Number of 96-Well Plates per Run	9
Average Number of Samples per Plate	80
Number of Samples per Run	720
Operation Time per Day	12 hrs
Number of 96-Well Plates per 12-Hour Shift	72
Total Number of Samples per 12-Hour Shift	5,760

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