Corning® Lambda™ EliteMax Benchtop Pipettor for ELISA Workflows

Application Note

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Introduction

Enzyme-linked immunosorbent assays or ELISAs are an essential analytical tool for detecting and quantifying molecules in solution[1] which is why they are frequently used in drug discovery, diagnostic tests, and quality control applications[2]. Running an ELISA requires multiple and tedious liquid handling steps. From adding proteins or antibodies to multiple washes to remove unbound components to the addition of the detection agent requires a tremendous amount of liquid manipulations. Here we demonstrate use of the Corning Lambda EliteMax benchtop automated pipettor to easily perform the numerous liquid handling steps needed for an indirect ELISA.

Materials and Methods

All liquid addition and removal steps were performed using plate-to-plate transfer protocols at medium speed and Axygen® 96-well tips (Corning FX-250-L-R). 150 µL of serially diluted recombinant human insulin (MP Biomedicals 193900), with a starting concentration of 0.4 µg/mL, from an 8-channel trough (Corning RES-MW8-HP-SI) were added across each well of 3 Corning 96-well clear flat bottom polystyrene high bind microplates (Corning 9018). Microplates were then sealed using Corning 96-well microplate aluminum sealing tape (Corning 6570) and stored at 2-8°C overnight prior to use. When ready, plates were removed from 2-8°C and allowed to warm to room temperature. Meanwhile, a 1X PBS wash buffer, containing 0.1% TWEEN® 20 (Sigma P2287), was prepared from a 10X phosphate buffered saline (PBS; Corning 46-013-CM) stock solution diluted with Type II water. The serially diluted insulin was removed and replaced with 150 µL of blocking buffer consisting of 3% bovine serum albumin (Sigma A9576-50ML) in 1X PBS wash buffer and incubated for 1 hour at room temperature. After the blocking buffer was removed, plates were washed 3 times with 200 µL of 1X PBS wash buffer by linking a plate-to-plate transfer protocol to remove liquid and a plate-to-plate transfer protocol to add 1X wash buffer. For ease of washing, Axygen single well reagent reservoirs with 96-bottom troughs (Corning RES-SW96-LP) were used for the 1X PBS wash buffer as well as for waste. Next, 100 µL of 0.76 µg/mL Mouse IgG1 insulin monoclonal antibody (ThermoFisher MA1-83252), diluted in 1X PBS wash buffer, was added to each well and incubated at room temperature for 1 hour. Plates were then washed three additional times as previously described. After washing the wells of unbound primary antibody, 150 µL of 0.5 µg/mL goat anti-mouse IgG (H+L) poly-HRP secondary antibody, HRP (ThermoFisher 32230) diluted in 1X PBS wash buffer was added to each well and incubated at room temperature for 1 hour. Microplates were washed 3 times after secondary antibody incubation following the previously described method. Finally, 100 µL of 1:1 mixed TMB 2-Component Microwell Peroxidase Substrate kit (SeraCare 5120-0047) was added to each well and incubated for approximately 5 minutes at room temperature. This color changing reaction was stopped with the addition of 100 µL per well of Stop solution (TMB 5150-0021) as per manufacturer's protocol. The change in color in the wells were then read on the Envision® multimode plate reader (PerkinElmer 2105-0010) at 450 nm.

Results and Discussion

Use of automation for repetitive processes can speed up assay workflows and reduce ergonomic risk but assay robustness and repeatability must not be compromised during implementation. Three independent ELISA assays were performed (n = 12) fully utilizing the Lambda EliteMax for all liquid handling tasks resulting in low intra-plate and inter-plate variability (Figure 1).

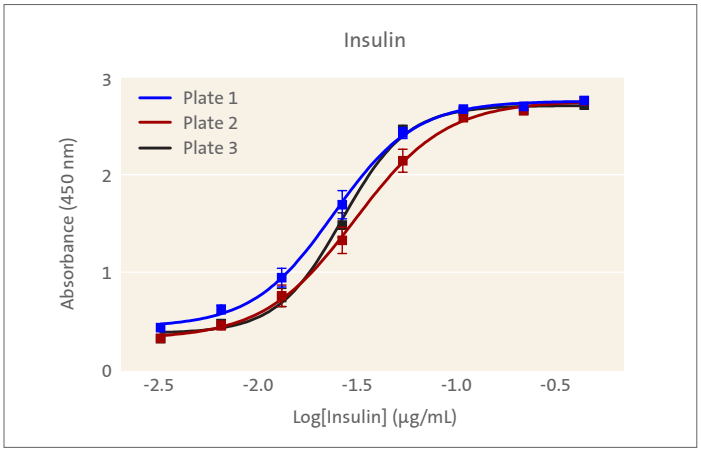

Figure 1. Dose dependent insulin binding achieved from ELISA automated with Corning Lambda EliteMax benchtop pipettor. Dose-dependent insulin binding curves generated from 3 microplates shown with mean and standard error. N = 12 per concentration. Average EC_{50} from 3 studies is 0.025.

Conclusions

ELISAs can be very labor-intensive and time-consuming due to numerous liquid addition and removal steps. Automating this process can save time, reduce intra-plate variability, and improve end user fatigue by reducing manual pipetting and improving ergonomics. Here, we demonstrated use of the Corning Lambda EliteMax benchtop pipettor to fully automate liquid handling steps for an indirect ELISA.

References

1. Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides 72 (2015): 4-15.
2. Hosseini S. et al. General overviews on applications of ELISA. Enzyme-linked Immunosorbent Assay (ELISA). Springer, Singapore, 2018. 19-29.

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