

ELISA workflows just got easier

With Instant ELISA kits, E1-ClipTip electronic pipette, and Wellwash Versa Microplate Washer.

Since the 1970s, the enzyme-linked immunosorbent assay (ELISA) has served as the mainstay for single-target protein quantitation, despite several cumbersome steps in its workflow. Specifically, assay steps associated with preparation, incubation, and washing can be vastly improved using innovative tools of the trade developed by Thermo Fisher Scientific. Here we describe three tools designed to simplify ELISA protocols.

Simplify preparation and incubation steps

Invitrogen™ Instant ELISA™ Kits are a family of immunoassays that have a condensed workflow format designed to alleviate the inconveniences of multiple preparation and incubation steps (Table 1). In conventional ELISA kits, the precoated plates provide only the capture antibody, to which the sample and detection reagents must be added sequentially. In contrast, Instant ELISA Kits come with ready-to-use plates that contain all necessary assay components—including capture antibody and lyophilized detection antibody, streptavidin-HRP, and sample diluent. These prepared plates greatly reduce pipetting time and enable a simple one-wash protocol (Table 1). Furthermore, serially diluted, lyophilized protein standards are provided in additional strip wells, eliminating the need for plate setup or serial dilutions to create a standard curve and leaving less room for error.

Table 1. Comparison of workflows for conventional ELISAs vs. Instant ELISA Kits.

17 steps for conventional ELISA kits	7 steps for Instant ELISA Kits
1. Washing of coated plates	
2. Reconstitution of standard proteins	
3. Addition of sample diluent to standard wells	
4. Titration of standard curve	1. Rehydration of plate
5. Addition of sample diluent	
6. Sample addition	2. Sample addition
7. Dilution of biotin conjugate	
8. Addition of biotin conjugate	
9. Incubation	3. Incubation
10. Preparation of streptavidin-HRP conjugate	
11. Washing	
12. Addition of streptavidin-HRP conjugate	
13. Incubation	
14. Washing	4. Washing
15. Addition of TMB substrate	5. Addition of TMB substrate
16. Addition of stop solution	6. Addition of stop solution
17. Calculation of results	7. Calculation of results

Execute a preprogrammed protocol

Rehydrating standards, introducing samples, and adding reagents can be simplified using a preprogrammed pipetting protocol that can be

A



B

Add	Step No.	Step	Parameters
	1	Notes	Add 150µL of distilled water to Standard and Blank wells
	2	Mix	1200 µL mix 10 s (mix 10 µL)
	3	Mix	100 µL mix 8 s (mix 10 µL)
	4	Purge	10 speed
	5	Load	Load start step 2 Copies 2
	6	Notes	Add 50µL of distilled water to Sample wells
	7	Mix	600 µL mix 10 s (mix 10 µL)
	8	Mix	100 µL mix 8 s (mix 10 µL)
	9	Purge	10 speed
	10	Transfer	
	11	Notes	Add 50µL of Sample in duplicate and mix 3 total different Samples
	12	Mix	100 µL mix 8 s (mix 10 µL)

C

1. Add 150 µL of distilled water to Standard and Blank wells.
2. Add 100 µL of distilled water to Sample well.
3. Add 50 µL of Sample in duplicate and mix.
4. Cover plate and incubate for 3 hours on microplate shaker if available at 400 rpm.
5. Remove plate cover, empty wells, and wash the microwell strips 6 times with 400 µL Wash Buffer per well with thorough aspiration of microwell contents.
6. Pipette 100 µL of TMB Substrate Solution to all wells, including the blank.
7. Incubate the microwell strips at room temperature (18° to 25°C) for 10–30 minutes (avoid direct exposure to intense light).
8. Add 100 µL of Stop Solution to each well.

Figure 1. Preprogrammed pipetting tools. (A) The programmable Thermo Scientific™ E1-ClipTip™ Multichannel Pipette. (B) The Thermo Scientific™ My Pipette™ Creator app (available at apps.thermofisher.com, powered by our Connect cloud-based platform) makes it easy to access and create assay protocols. (C) An example of a protocol for an Invitrogen™ Instant ELISA™ Kit.

easily transferred to the Thermo Scientific™ E1-ClipTip™ Electronic Multichannel Pipette (Figure 1A), saving valuable time and minimizing potential errors. A preprogrammed pipetting protocol can be downloaded from the Thermo Scientific™ My Pipette™ Creator app (Figure 1B) to an E1-ClipTip pipette. Using the My Pipette Creator app, custom pipetting programs can be quickly created, edited, and then transferred to a E1-ClipTip pipette through a USB or, for some models, a wireless connection. Once programmed, the pipette will automatically step you through the protocol (Figure 1C) with concise and clear instructions, even prompting when it is time for the plate to be incubated.

Automate wash steps

During an ELISA protocol, wash steps are extremely important for decreasing background levels, which can impact both the variability and sensitivity of the assay. Manual wash steps with either squirt bottles or multichannel pipettes can be awkward and unwieldy, introducing another potential source of variation. The Thermo Scientific™ Wellwash™ Versa Microplate Washer (Figure 2) makes wash steps easier, more efficient, and



Figure 2. Automation of wash steps. Thermo Scientific™ Wellwash™ Versa Microplate Washer (Cat. No. 5165010).

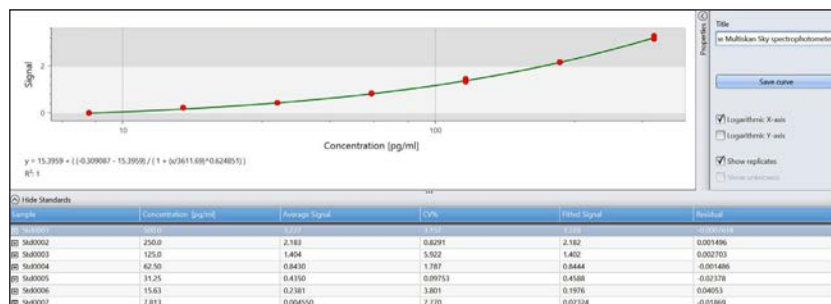


Figure 3. Standard curve data with an average CV of 3.3% using the TNF alpha Human Instant ELISA Kit. The Invitrogen™ TNF alpha Instant ELISA™ Kit (Cat. No. BMS223INST) was used according to the manufacturer’s protocol using a programmable Thermo Scientific™ E1-ClipTip™ Multichannel Pipette and the Thermo Scientific™ Wellwash™ Versa Microplate Washer (Cat. No. 5165050). Data were analyzed using the Thermo Scientific™ Multiskan™ Sky Microplate Spectrophotometer with Thermo Scientific™ SkanIt™ Software (Cat. No. 51119700).

more consistent. With programmable volumes and soak times, the Wellwash Versa instrument is designed for high performance and versatility and can be configured with a 2 x 8 head to accommodate the 2 x 8-well standard curve strip format.

Streamline ELISAs in your lab

The ELISA workflow can be significantly streamlined when combining these three time-saving tools, generating data with low coefficients of variation (CVs) (Figure 3). The Instant ELISA Kit format simplifies both the preparation and incubation steps and reduces hands-on time. Instead of tending to your plate every hour to perform washes, you have a 3-hour window to focus on something else before wrapping up the assay run. The My Pipette Creator app and E1-ClipTip electronic pipette make the steps in the ELISA workflow more efficient by reducing pipetting time and minimizing errors. And the Wellwash Versa Microplate Washer automates the single Instant ELISA wash step, providing consistency to help prevent incomplete washing and associated adverse outcomes, as well as convenience. To learn more about how these Thermo Fisher Scientific tools can provide an overall easier and more efficient ELISA workflow, go to thermofisher.com/elisa. ■

Product	Quantity	Cat. No.
TNF alpha Human Instant ELISA™ Kit	128 tests	BMS223INST
ClipTip™ 1250 Filtered Sterile Pipette Tips	Case of 768	94420813
E1-ClipTip™ Bluetooth™ Electronic Single-Channel Pipette, 15 to 1,250 µL	1 each	4670040BT
E1-ClipTip™ Bluetooth™ Electronic 8-Channel Pipette, 15 to 1,250 µL	1 each	4671100BT
Multiskan™ Sky Microplate Spectrophotometer, with touchscreen and cuvette	1 each	51119700
SkanIt™ Software for Microplate Readers, Research Edition	1 each	5187139
Wellwash™ Versa Microplate Washer, 2 x 8 model	1 each	5165010
Wellwash™ Versa Microplate Washer, 2 x 12 model	1 each	5165050