

Performing an ELISA with the VIAFLO 96/384 handheld electronic pipette

Introduction

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay that is commonly used to quantify and detect peptides, proteins, antibodies or hormones. Well to well and plate to plate consistency and reproducibility are key success factors for ELISAs, which consist of multiple pipetting steps that are time-consuming and tedious to perform manually. Using the VIAFLO 96/384 handheld electronic pipette can vastly improve ELISAs. The 96 channel pipetting head makes

the whole process faster, less error prone and much more reproducible compared to traditional single or multichannel pipettes by transferring the samples and reagents into all 96 wells of the plate at the same time. The VIAFLO 96/384 electronic pipette offers clear gains in productivity, consistency and reproducibility while remaining as easy to use as a traditional handheld pipette.

Key benefits:

- The VIAFLO 96/384 allows easy and rapid simultaneous transfer of all samples for optimal productivity, reproducibility, and massive time reduction.
- All the steps needed to run the ELISA protocol can be saved on the pipette as a custom program containing all the necessary settings to guide the user through the process via easy to follow prompts.
- The pipetting heights are saved to avoid any crashes and scratching of the coated surface.
- For higher throughput assays, multiple plates can be quickly filled using the Repeat Dispense mode of the VIAFLO 96/384.

Step-by-step procedure:

Experimental set-up

The following protocol shows an example set-up for performing a sandwich ELISA using the VIAFLO 96/384 handheld electronic pipette together with a three position stage (**Figure 1**). In this protocol, **position A** is dedicated to the GripTip box and the liquid waste reservoir, **position AB** to reagent reservoirs containing the various reagents and washing solutions required, and **position B** to the ELISA plate. A 96 channel pipetting head (10-300 µl) is used together with 300 µl Sterile, Filter, Low Retention GripTips. Customized VIALINK programs are provided for performing a sandwich ELISA with the VIAFLO 96/384.

Note: The VIALINK programs provided can be easily adapted to any other ELISA type, e.g. direct, indirect or competitive ELISAs, by just adding or removing steps from the protocols supplied.

VIAFLO 96/384



Three position stage

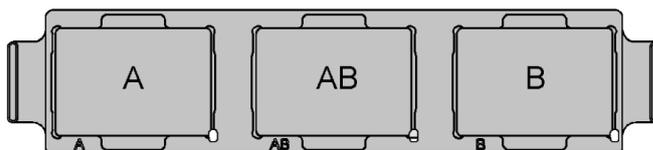


Figure 1: VIAFLO 96/384 with a three position stage

1. Coat the ELISA plate

STEP: Adding the capture antibody to coat the ELISA plate.

HOW TO: Place the capture antibody, prediluted in the coating buffer, into a 150 ml automation friendly reagent reservoir. Select and run the VIALINK E_REAGENTS program, then simply follow the instructions on the pipette. Pipette 100 μ l of the capture antibody into the ELISA plate and then incubate it.

Tips:

- A z-height limit is defined to ensure an optimal tip immersion depth, preventing both air entering into the tips during the aspiration step and the pipette tips from touching the bottom of the plate. We also recommend setting the Tip Align support strength to 3 for this application.
- To increase your throughput, the Repeat Dispense mode of the VIAFLO 96/384 electronic pipette can be used to rapidly prepare several plates at once. The VIALINK program E_REAGENTS_3 PL is an example of a multiple plate filling protocol.



Figure 2: The VIAFLO 96/384 is ideal for high throughput assays, quickly filling multiple plates using the Repeat Dispense mode.

2. Block the plate

STEP: Blocking the ELISA plate's non-specific binding sites.

HOW TO: Select the VIALINK program E_REAGENTS to remove the coating buffer from the plate, then use the E_WASH program to wash the plate three times with 200 µl of washing buffer from a 300 ml automation friendly reagent reservoir. Firmly blot the plate against clean paper towels. To block the ELISA plate, select the corresponding E_BLOCK program and transfer 300 µl of the blocking buffer from a 150 ml automation friendly reservoir into the plate before incubation.

Tips:

- All wells are treated simultaneously and in the same way. This ensures not only the well to well consistency of the assay, but also the reproducibility from plate to plate.
- The z-height is set 1 mm above the bottom of the plate, preventing the tips from scratching the coated surface, which can lead to inaccurate or inconsistent assay results.

3. Add the controls and samples

STEP: Adding the diluted samples and controls to the ELISA plate.

HOW TO: Select and run the E_BLOCK program to remove the blocking buffer, then wash the plate using the E_WASH program. In the current example (see **Figure 3**), the controls and samples are dispensed into a 96 well plate. The E_REAGENTS program allows rapid, simultaneous transfer of all samples and controls into the ELISA plate before incubation.

Tips:

- Adding all the samples at once ensures optimal productivity and reproducibility of the ELISA.
- If your samples are stored in microcentrifuge tubes, using a VOYAGER 8 channel 300 µl adjustable tip spacing pipette allows you to quickly reformat them from the tubes to the plate at the simple touch of a button, while reducing transcription errors when compared to a single channel pipette.
- ELISA buffers often contain surfactants, such as Tween 20. Using Low Retention GripTips reduces the residual volume in the tips for maximum sample recovery.

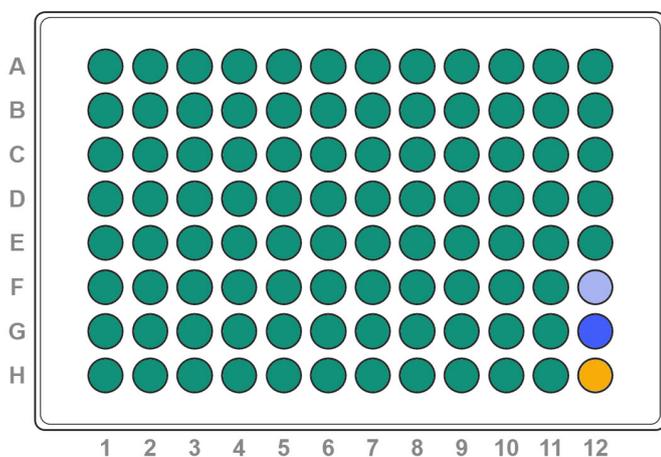


Figure 3: Example of an ELISA plate set up using the VIAFLO 96/384: samples (green); blank (pale blue); positive control (dark blue); and negative control (orange).



Figure 4: The use of a VOYAGER adjustable tip spacing pipette makes sample reformatting quick and easy.

Pipetting buffers with Low Retention GripTip pipette tips

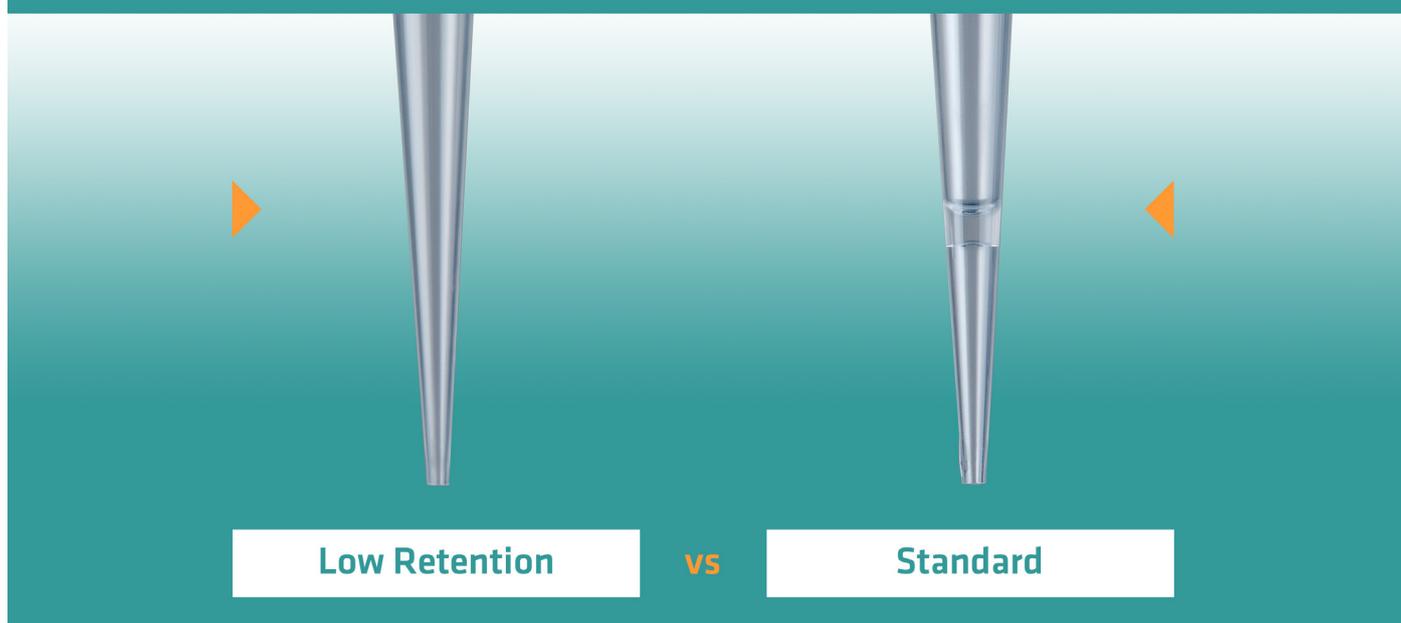


Figure 5: The image highlights the advantages of using Low Retention GripTips (left) versus standard GripTips (right) when pipetting buffers containing surfactants.

4. Add the detection antibody

STEP: Adding the diluted detection antibody to the ELISA plate.

HOW TO: After incubation, select the E_REAGENTS program to remove the sample buffer, then use E_WASH to wash the plate three times. Place the detection antibody into a 150 ml automation friendly reagent reservoir on **position AB**. Run the E_REAGENTS program to rapidly transfer the detection antibody into the ELISA plate before incubation.

5. Add the enzyme conjugate

STEP: Adding the enzyme conjugate to the ELISA plate.

HOW TO: Repeat step 4 to add the enzyme conjugate. Incubate the plate.

6. Add the substrate

STEP: Adding the TMB substrate and incubating the plate until the color develops sufficiently.

HOW TO: Remove the buffer then thoroughly wash the plate six times before adding 100 μ l of TMB substrate to the ELISA plate. Incubate the plate at room temperature: the color of the solution changes from transparent to blue in wells where the samples have reacted with the antibodies. The color intensity depends on the sample concentration (see example in **Figure 6**).



Figure 6: Example of a sandwich ELISA plate after incubation with TMB substrate; positive (blue) and negative (clear) reaction of the samples with the antibodies. The color intensity directly correlates with the sample concentration.

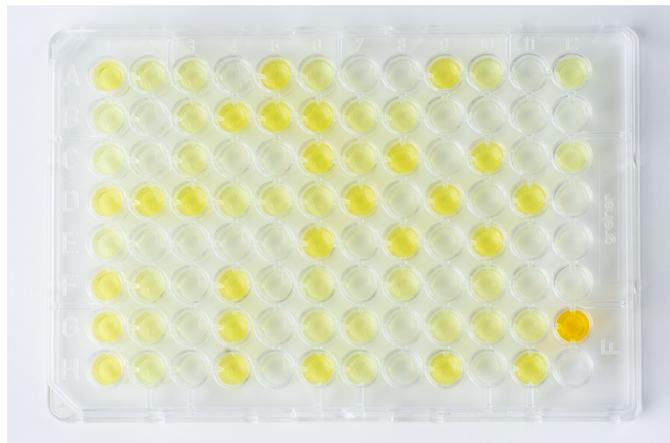


Figure 7: Example of a sandwich ELISA plate after incubation with TMB substrate and addition of the stop solution; positive (yellow) and negative (clear) reaction of the samples with the antibodies. The color intensity directly depends on the sample concentration.

7. Stop the reaction

STEP: Adding the stop solution to the plate before detection.

HOW TO: Use the VIALINK E_REAGENTS program to add the stop solution to the plate; the color changes from blue to yellow in wells where the samples have reacted with the antibodies (see **Figure 7**). The plate is now ready for detection.

Remarks

Partial tip load:

If your particular ELISA doesn't require processing of 96 samples, the VIAFLO 96/384 is able to work with any number of tips loaded, giving you the benefit of simultaneous and precise dispensing of a smaller number of samples.

For better ergonomics:

The instrument can also operate on its own, reducing user interaction, which in turn improves ergonomics and reproducibility. This also makes the VIAFLO 96/384 ideal for use in tight spaces, such as under a laminar flow cabinet.

Conclusion

- The VIAFLO 96/384 allows all wells of a 96 well plate to be treated at the same time, offering optimal consistency and reproducibility of the results from well to well, and plate to plate.
- Thanks to its unique operating concept, the VIAFLO 96/384 electronic pipette is as easy to use as any traditional handheld pipette.
- Optimized pipetting settings ensure easy and fast sample and reagent transfers, while avoiding the risk of damaging the coated surface of the plate.
- The VIAFLO 96/384 electronic pipette is adaptable to your needs, either by working with a reduced number of tips for partial plate processing or by working on its own for improved ergonomics, especially when used in tight spaces, such as a laminar flow cabinet.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	6001/6031	VIAFLO 96 or 384 handheld electronic pipette (base unit)	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#downloads
INTEGRA Biosciences	6230	Three position stage for 96 and 384 well plates	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6123	96 channel pipetting head 300 µl	https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96384#parts-and-numbers
INTEGRA Biosciences	6307, 6305, 6327	300 ml automation friendly reagent reservoir	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs#parts-and-numbers
INTEGRA Biosciences	6303, 6301, 6317	150 ml automation friendly reagent reservoir	https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs#parts-and-numbers
INTEGRA Biosciences	6535	300 µl Sterile, Filter, Low Retention GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
INTEGRA Biosciences	4743	VOYAGER 8 channel 300 µl electronic pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager#parts-and-numbers

INTEGRA Biosciences AG
 7205 Zizers, Switzerland
 T +41 81 286 95 30
 F +41 81 286 95 33
info@integra-biosciences.com

INTEGRA Biosciences Corp.
 Hudson, NH 03051, USA
 T +1 603 578 5800
 F +1 603 577 5529
info-us@integra-biosciences.com

INTEGRA Biosciences Deutschland GmbH
 35444 Biebertal, Deutschland
 T +49 6409 81 999 15
 F +49 6409 81 999 68
info-de@integra-biosciences.com

INTEGRA Biosciences SAS
 95062 Cergy-Pontoise Cedex 1, France
 T +33 (0)1 34 30 76 76
 F +33 (0)1 34 30 76 79
info-fr@integra-biosciences.com

INTEGRA Biosciences Ltd.
 Egham, Surrey TW20 9EY, UK
info-uk@integra-biosciences.com