Performing an ELISA with the ASSIST PLUS pipetting robot

Introduction

The enzyme-linked immunosorbent assay (ELISA) is a standard method used to detect and quantify peptides, proteins, antibodies or hormones in a sample. It consists of multiple repetitive steps that are time consuming and tedious to perform manually. The ASSIST PLUS pipetting robot allows this process to be automated, which not only increases the reproducibility of your results, but also gives you more time to focus on your science. Any VIAFLO or VOYAGER electronic pipette can be automated using the ASSIST

PLUS; the VOYAGER adjustable tip spacing pipette enables reformatting of samples from one labware type to another in the blink of an eye. All the steps needed to run an ELISA are saved on the pipette as a VIALAB program – the smart and easy-to-use pipetting automation software of the ASSIST PLUS. Simply place the labware on the deck, choose the program corresponding to the ELISA step and let the ASSIST PLUS do the work.

Key benefits:

- Optimal pipette settings including tip immersion depth, pipetting speeds and angles – maximize the consistency and reproducibility of the ELISA.
- The full automation capability of the ASSIST PLUS frees highly valuable time that you can use for more important tasks.
- VOYAGER and VIAFLO electronic pipettes, in combination with the ASSIST PLUS, provide unmatched pipetting ergonomics.
- Repeat Dispense and Multi Aspirate steps can be used

Step-by-step procedure:

Experimental set-up

The ASSIST PLUS is used to perform a sandwich ELISA. The pipetting robot operates a VOYAGER 8 channel 1250 µl electronic pipette with 1250 µl Sterile, Filter, Low Retention GripTips. The use of Low Retention GripTips guarantees optimal liquid recovery when pipetting ELISA buffers that contain surfactants, such as Tween 20.

Below is an example set-up for a sandwich ELISA with a standard curve and 24 samples in triplicate. The pipetting programs are prepared with the VIALAB software. The protocol is divided into eight programs that guide the user through the eight steps of the ELISA.

for fast dispense and removal of reagents to speed up the process. The automatic Tip Change ensures assay contamination is avoided.

- The ASSIST PLUS pipetting robot is perfectly adapted to handle different plate layouts, increasing the flexibility of your work depending on your needs.
- Various sample input tubes can be used. The samples are easily transferred to the assay plate using multichannel and adjustable tip spacing pipettes, increasing the assay productivity while avoiding reformatting errors.



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Overview of the sandwich ELISA steps and corresponding programs:

Program 1: Coat the plate (1_E_Coating)
Program 2: Block the plate (2_E_Blocking)
Program 3: Prepare the samples (3_E_Sample_Preparation)
Program 4: Add the samples (4_E_Sample_Addition)
Program 5: Add the detection antibody (5_E_Detection_Antibody)
Program 6: Add the enzyme conjugate (6_E_Enzyme_Conjugate)
Program 7: Add the substrate (7_E_Substrate)
Program 8: Stop the reaction (8_E_Stop_Solution)

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, in a 10 ml polypropylene multichannel reagent reservoir. Select and run the first VIALAB program, 1_E_Coating. The pipette automatically transfers 100 µl of the capture antibody into the ELISA plate using the Repeat Dispense mode. The plate is ready to be incubated.



Figure 1: The set-up for program 1_E_Coating. Position A: 10 ml polypropylene multichannel reagent reservoir containing the capture antibody. Position B: ELISA plate.

2. Block the plate

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

HOW TO: Select the VIALAB program 2_E_Blocking and set up the deck with the required labware, as indicated in **Figure 2**.

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Figure 2: The set-up for program 2_E_Blocking. Position A: 8 row polypropylene reservoir containing the washing buffer (green) and the blocking buffer (pink). Position B: ELISA plate. Position C: 300 ml automation friendly reservoir, used to collect the liquid waste.



Figure 3: The image highlights the advantages of using Low Retention GripTips (left) versus standard GripTips (right) when pipetting buffers containing surfactants.

The program incorporates all the necessary pipetting steps, including removing of the coating buffer from the plate followed by washing three times with 200 µl of the washing buffer. In our example, we included a 15 second incubation time, which can be easily adjusted to your protocol using the VIALAB software. The use of the Repeat Dispense and Multi Aspirate modes speeds up this fully automated process. At the end of the washing steps, the user is prompted to blot the plate against clean paper towels – the only manual step of this ELISA protocol. After confirming that this step has been completed, the ASSIST PLUS pipetting robot continues by adding the blocking buffer into the ELISA plate using the Repeat Dispense mode. Finally, the pipette informs the user that the plate is ready for incubation.

Tips:

- All wells are treated in the same way. This is reproducible from plate to plate, ensuring the consistency of your results.
- Setting a height limit 1 mm above the bottom of the ELISA plate guarantees that the pipette tips will never damage the coating surface, offering a safe and reproducible protocol from plate to plate, and avoiding inconsistencies or inaccurate results.
- Using an eight row polypropylene reservoir allows the deck set-up to be optimized, enabling several buffers to be handled on just one deck position. This means that there is no need to change the labware during a protocol, allowing fully automated processing of the ELISA plate.
- Using Low Retention GripTips is optimal when pipetting buffers that contain surfactants, such as Tween 20. They ensure the greatest liquid recovery in comparison with standard tips (see Figure 3).

3. Prepare your samples

STEP: Diluting your samples 1:10.

HOW TO: In this example, centrifuged blood samples are stored in EDTA collection tubes placed in an INTEGRA rack. The plasma is diluted 1:10 with the dilution buffer. The deck layout is shown in **Figure 4**.



Figure 4: The set-up for program 3_E_Sample_Preparation. **Position A:** 24 EDTA collection tubes containing whole blood in an INTEGRA rack (green). **Position B:** 150 ml automation friendly reagent reservoir filled with dilution buffer. **Position C:** INTEGRA rack containing 24 empty 1.5 ml microcentrifuge tubes for the diluted samples (pink). The first column of microcentrifuge tubes is dedicated to the blank and serial dilutions of the ELISA standard for the standard curves.

Select and run the 3_E_Sample_Preparation program. The pipette automatically fills the microcentrifuge tubes with 900 μ l of the dilution buffer. This is followed by transfer of the plasma samples from the EDTA tubes to the microcentrifuge tubes, and careful and thorough mixing. The samples are then ready to be used.

Tips:

- Using a VOYAGER adjustable tip spacing pipette together with the ASSIST PLUS allows automatic and error-free sample reformatting.
- The ASSIST PLUS pipetting robot ejects and loads the tips automatically, eliminating any risk of sample cross-contamination.

4. Add your controls and samples

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

HOW TO: Prepare the deck of the ASSIST PLUS as indicated in **Figure 5**.



Figure 5: The set-up for program 4_E_Sample_Addition. **Position A:** 8 row polypropylene reservoir containing the washing buffer (green). Rows 4 to 7 are used to dispense the liquid waste. **Position B:** ELISA plate. **Position C:** INTEGRA rack for 1.5 ml microcentrifuge tubes. The first pink column contains the blank and the ELISA standard, serially diluted for the standard curve. The three other columns in pink contain the diluted plasma samples.

Select and run the 4_E_Sample_Addition program to remove the blocking buffer and subsequently wash the plate. In our example, each sample is added to the ELISA plate in triplicate. The ASSIST PLUS pipetting robot uses the Repeat Dispense mode to transfer the triplicate samples into the plate, replacing the pipette tips before aspirating the next series of samples.

Tips:

• Each pipetting step is done in exactly the same way, ensuring the reproducibility of the assay from row to row, and plate to plate.

5. Add the detection antibody

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

HOW TO: After incubation, prepare the deck as indicated in **Figure 6** and select program 5_E_Detection_Antibody. The addition of the detection antibody is performed automatically by the ASSIST PLUS. Incubate the plate again.



Figure 6: The set-up for program 5_E_Detection_Antibody. **Position A:** 8 row polypropylene reservoir containing the washing buffer (green) and the detection antibody (pink). **Position B:** ELISA plate. **Position C:** 300 ml automation friendly reservoir, for waste collection.

6. Add the enzyme conjugate

STEP: Adding the enzyme conjugate to the ELISA plate.

HOW TO: Set up the ASSIST PLUS deck as shown in **Figure 7**. Select program 6_E_Enzyme_Conjugate, which includes the removal of the previous solution, the three washing steps, and the addition of the enzyme conjugate to the ELISA plate. The plate is ready for incubation.



Figure 7: The set-up for program 6_E_Enzyme_Conjugate. **Position A:** 8 row polypropylene reservoir containing the washing buffer (green) and the enzyme conjugate (pink). **Position B:** ELISA plate. **Position C:** 300 ml automation friendly reservoir, to collect the liquid waste.

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Figure 8: The set-up for program 7_E_Substrate. **Position A:** 8 row polypropylene reservoir containing the washing buffer (green) and the TMB substrate (pink). **Position B:** ELISA plate. **Position C:** a 300 ml automation friendly reservoir, for waste collection.



Figure 9: Example of a sandwich ELISA plate after incubation with TMB substrate, showing positive (blue) and negative (clear) reactions of the triplicate samples with the antibodies. The color intensity directly depends on the sample concentration, as highlighted in the first three columns, which correspond to the standard curves.

Select and run program 7_E_Substrate. The ASSIST PLUS removes the previous buffer then washes the plate six times before adding the TMB substrate. Incubate the plate at room temperature until the color is sufficiently developed. The color of the solution changes from transparent to blue in wells where the samples have reacted with the antibodies. The color intensity is dependent on the sample concentration (see **Figure 9**).

Tips:

- The pipetting robot automatically processes the plate, regardless of the multiple and repetitive pipetting steps, freeing up time for you to concentrate on other tasks.
- The ASSIST PLUS tells you when to add the TMB substrate into the corresponding row of the reservoir, preventing the photosensitive substrate from being exposed to light for too long a period of time.

Application Note

8. Stop the reaction

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

HOW TO: Select and run the final program, 8_E_Stop_ Solution (see **Figure 10** for the deck set-up).



Figure 10: The set-up for program 8_E_Stop_Solution. The deck set-up is identical to that of step 7, except that the stop solution is added to the 8th row of the 8 row polypropylene reservoir (green).



Figure 11: Example of a sandwich ELISA plate after incubation with TMB substrate and addition of the stop solution, showing positive (yellow) and negative (clear) reactions of the triplicate samples with the antibodies. The color intensity directly depends on the sample concentration, as highlighted in the first three rows, which correspond to the standard curves.

The ASSIST PLUS adds the stop solution to the sample triplicates in the plate; the color changes from blue to yellow in the wells where the samples reacted with the antibodies (see **Figure 11**). The plate is now ready for detection.

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Remarks

Partial plate: If your particular ELISA doesn't require processing of 96 samples, the ASSIST PLUS is able to work with any number of columns. Simply adapt the VIALAB program to fit your need.

VIALAB software: The VIALAB programs can be easily adapted to your specific labware and protocols.

Conclusion

- ELISAs can be fully automated using the ASSIST PLUS pipetting robot, offering users increased walk-away time.
- Optimized pipetting settings and tip immersion, together with the use of Low Retention GripTips, guarantee the consistency and reproducibility of the ELISAs.
- Using the ASSIST PLUS pipetting robot allows
 various sample tube types and multiple reagents to be

accommodated on the deck, for improved productivity and unrivaled flexibility.

- Automatic Tip Change avoids any assay contamination while using the Repeat Dispense and Multi Aspirate modes whenever possible speeds up the process.
- Thanks to the VIALAB software, the pipetting programs can be easily adapted to specific protocols and labware.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/ pipetting-robots/assist-plus
INTEGRA Biosciences	4724	VOYAGER 8 channel 1250 µl electronic pipette	https://www.integra-biosciences.com/global/en/ pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6545	1250 μl Sterile, Filter, Low Retention GripTips	https://www.integra-biosciences.com/global/en/ griptip-selector-guide
INTEGRA Biosciences	4337, 4336	10 ml reagent reservoir, polypropylene	https://www.integra-biosciences.com/switzerland/ en/reagent-reservoirs/multichannel-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	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	6371	8 row polypropylene reservoirs	https://www.integra-biosciences.com/global/en/ reagent-reservoirs/automation-friendly-reagent- reservoirs

INTEGRA Biosciences	4540	Rack for 1.5 ml microcentrifuge tubes – 1500 μl	https://www.integra-biosciences.com/switzerland/en/ pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	4543	Rack for 6 ml EDTA blood test tubes (12 x 100 mm) 6000 µl	https://www.integra-biosciences.com/switzerland/en/ pipetting-robots/assist-plus#parts-and-numbers
Greiner Bio-One	762070	Strip Plate, 12 x F8, PS, F-bottom, clear, Microlong [®] , Med. Binding	https://shop.gbo.com/de/switzerland/products/ bioscience/immunologie-hla/96-well-elisa- streifenplatten/8-well-streifen-platten/762070. html?_ga=2.123933386.159100769.1581674193- 1363954463.1581674193
Greiner Bio-One	456038	VACUETTE [®] TUBE 6 ml K3E K3EDTA 13 x 100 lavender cap – black ring, non-ridged	https://shop.gbo.com/en/switzerland/products/ preanalytics/venous-blood-collection/vacuette-tube/ edta/k3e-k3edta/456038.html

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