

Performing an endotoxin test (ENDOZYME® II GO) using a recombinant factor C (rFC) assay and an automated pipetting robot

Introduction

The ENDOZYME® II GO kit was developed by bioMérieux. It includes a novel 96 well microplate (GOPLATE™) pre-loaded with the required Control Standard Endotoxin (CSE) concentrations for a standard curve, as well as Positive Product Controls (PPC). The ASSIST PLUS pipetting

robot provides a solution to minimize hands-on time and further optimize the assay workflow, without the need for dedicated personnel. Automation of the protocol with the ASSIST PLUS helps to prevent pipetting errors and ensures outstanding data reproducibility.

Key benefits:

- The ASSIST PLUS reduces hands-on time for preparing a full 96 well GOPLATE from 10 minutes to less than two minutes.
- Optimal pipette settings, such as fixed pipetting heights, angles and speeds, lead to increased reliability, precision and accuracy.
- An on-deck heating solution – an INHECO microplate heater – can be placed on the ASSIST PLUS deck in order to further streamline the workflow and increase the throughput by 33 %.
- The ASSIST PLUS ensures process security to minimize pipetting errors.

Step-by-step procedure:

The ASSIST PLUS is used to perform an ENDOZYME II GO endotoxin test from bioMérieux, using a pipetting program generated by the VIALAB software.

Note: Prepare the assay reagent manually in advance by combining enzyme, substrate and assay buffer, according to the manufacturer's protocol (ENDOZYME II GO, bioMérieux).

Experimental set-up:

Deck position A: 25 ml multichannel reservoir – filled with 12 ml assay reagent (**Figure 1**, in blue).

Deck position B: 96 well flat bottom (GOPLATE) placed on a microplate heater (HeatPAC, INHECO).

Deck position C: tube rack for 15 ml centrifuge tubes – with 5000 µl of sample (**Figure 1**, in green). Alternatively, a custom rack for ENDOGRADE® Glass Test Tubes can be used with 1000 µl of sample.

ASSIST PLUS



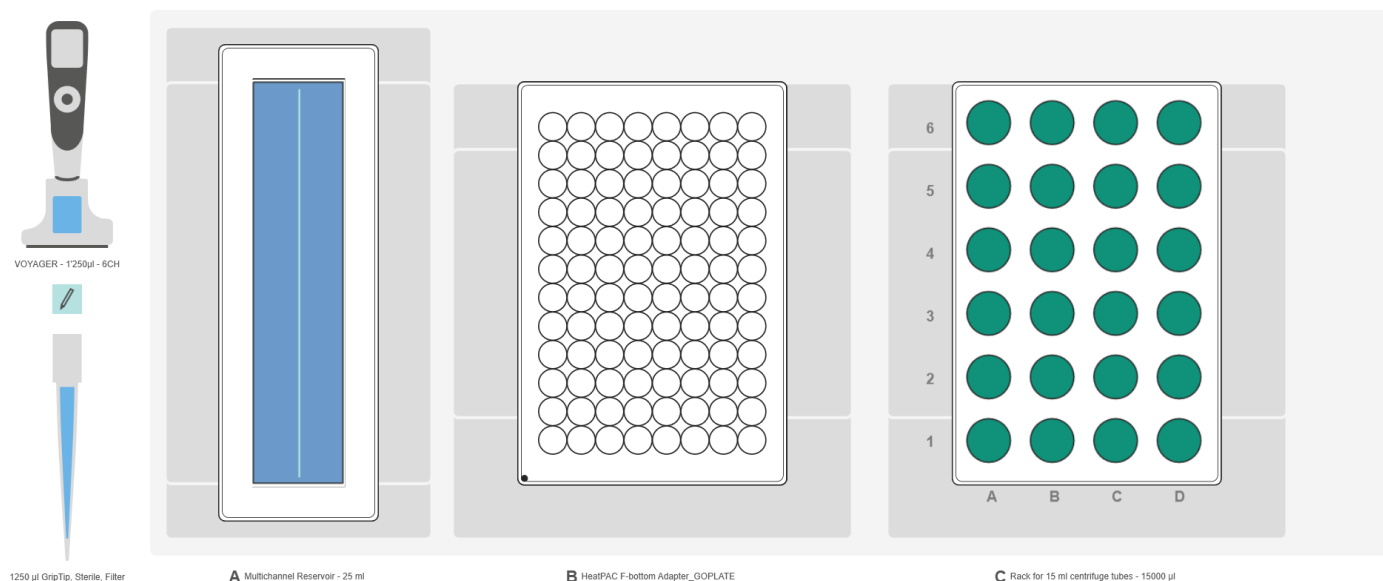


Figure 1: Deck set-up.

1. Add water and samples to the dedicated wells

STEP: Distribute samples into the 96 well GOPLATE.

HOW TO: Use the Repeat Dispense mode of the ASSIST PLUS to equally distribute samples from the tube rack (**position C**) into the dedicated wells of the GOPLATE (**position B**).

Tip: Insert a pre- and post-dispense step to improve accuracy and precision while pipetting. The recommended pre- and post-dispense volume is 3-5 % of the nominal volume of the pipette. Insert an air gap to eliminate any risk of contamination. Perform a tip touch to the wall of the well to remove droplets that may cling to the end of the pipette tips. The pipetting height is set to 65 mm so that the tip does not touch the solution.

2. Heat the GOPLATE containing the samples

STEP: Incubate your samples on the microplate heater (INHECO).

HOW TO: Start the incubation of the plate on the INHECO microplate heater at the appropriate temperature and incubate the plate for five minutes (ENDOZYME II GO, bioMérieux). If there is no microplate heater available on the deck, the plate can be manually removed and incubated on an external microplate heater or in the microplate fluorescence reader.

3. Add the assay reagent

STEP: Add the assay reagent to the dedicated wells of the 96 well GOPLATE.

HOW TO: Use the Repeat Dispense mode to transfer assay reagent from **position A** into the GOPLATE (**position B**).

Tip: Adjusting the pipette settings (tip travel, tip touch and pipetting height) increases the precision and accuracy of pipetting. The tip touch automatically touches the tip to the side of the well after each dispense to remove droplets clinging to the tip end. In this protocol, the tip touch of the samples and assay reagent is set to the opposite well sides to avoid the risk of cross-contamination between samples.

4. Run the assay in a fluorescence reader

STEP: Determine the endotoxin content with a microplate fluorescence reader.

HOW TO: After adding the assay reagent, place the GOPLATE immediately in a fluorescence microplate reader and start your readings according to the manufacturer's protocol (ENDOZYME II GO, bioMérieux).

Conclusion

- The ENDOZYME II GO kit can be easily automated with the ASSIST PLUS.
- Using an on-deck heating solution reduces manual handling and increases the throughput by 33 %.
- Accuracy and precision of the semi-automated workflow is equal to or higher than that of manual pipetting.
- Using the semi-automated process approach described in this application note offers higher efficiency and throughput of recombinant factor C (rFC) endotoxin testing.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4764	VOYAGER 6 channel 1250 µl electronic pipette	https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager#parts-and-numbers
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus#parts-and-numbers
INTEGRA Biosciences	6445	1250 µl Sterile, Filter GripTips	https://www.integra-biosciences.com/global/en/griptip-selector-guide
INTEGRA Biosciences	4311, 4312	25 ml multichannel reagent reservoir	https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	4542	Rack for 15 ml centrifuge tubes, 4x6 tubes	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INTEGRA Biosciences	128011	Custom Rack for ENDOGRADE Glass Test Tubes	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
bioMérieux	890031	ENDOZYME II GO	https://biomerieuxdirect.com/industry/c/ENDOZYME®-II-GO/p/890031
bioMérieux	800050	ENDOGRADE Glass Test Tubes	https://biomerieuxdirect.com/industry/c/ENDOGRADE®-Glass-Test-Tubes/p/800050
INTEGRA Biosciences	128151	INHECO CPAC/HeatPac SBS adapter kit	https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus
INHECO	7900046	HeatPAC	https://www.inheco.com/products/lab-automation/heater-cooler/heat.html
INHECO	7900016	Flat bottom adapter and positioning frame	https://www.inheco.com/products/lab-automation/heater-cooler/heat.html
INHECO	7900001	Single TEC Control (STC) with black slot module	https://www.inheco.com/products/lab-automation/heater-cooler/heat.html

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