Application Note

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3 simple and proven automation protocols for serial dilutions on the ASSIST PLUS pipetting robot

Introduction

Serial dilution – a reduction in concentration by a constant dilution factor – is a common approach for screening-related applications, such as determining minimum inhibitory concentrations (MIC) in drug discovery, calculating the most probable numbers (MPN) in microbiology, and performing general nucleic acid quantifications in molecular biology. Although it is a simple technique, poor liquid handling during interdependent dilution steps can cause error propagation and accumulation. Thorough mixing is therefore crucial, but this puts a lot of strain on the thumb, which increases the risk of repetitive strain injuries. In addition, performing serial dilutions regularly can be a time-consuming process.

This application note describes the simplest way to do serial dilutions, using the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot to gain more walk-away time. The protocols provided outline the optimal settings to ensure reliable results when diluting analytes in water. For further information about modifying key parameters to suit varying conditions, see INTEGRA's comprehensive guide to performing serial dilution.

Key benefits:

- Proven serial dilution protocols, with optimal settings for the VOYAGER on the ASSIST PLUS, guarantee uniform pipetting and mixing.
- The VOYAGER offers flexible automated serial dilutions across various tubes and plates, as well as the ability to switch pipette volumes seamlessly while maintaining the same protocol.
- INTEGRA's electronic pipettes prevent thumb strain during liquid handling steps and, together with the ASSIST PLUS, enable risk-free handling of hazardous samples.
- The ASSIST PLUS gives users additional hands-free time, eliminating time-consuming manual procedures.
- These efficient liquid handling solutions support 2-, 5- and 10-fold serial dilutions, with dynamic mixing volumes ensuring homogeneity of analytes.
- Simplified workflows are achieved with VIALAB's serial dilution protocol, which includes specific mixing parameters for managing poorly soluble analytes.

Overview: How to do serial dilution with the ASSIST PLUS

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This application note demonstrates how to perform serial dilution of tartrazine in water with an 8 channel 125 µl VOYAGER on the ASSIST PLUS.

Experimental set-up

The ASSIST PLUS, together with the 125 µl 8 channel VOYAGER and 125 µl sterile, filter GRIPTIPS® pipette tips, automates complete serial dilutions in 1 program consisting of 3 steps (**Figure 1**):

1. Transfer diluent to target plate
2. Transfer analyte to target plate
3. Serial dilution of analyte within target plate

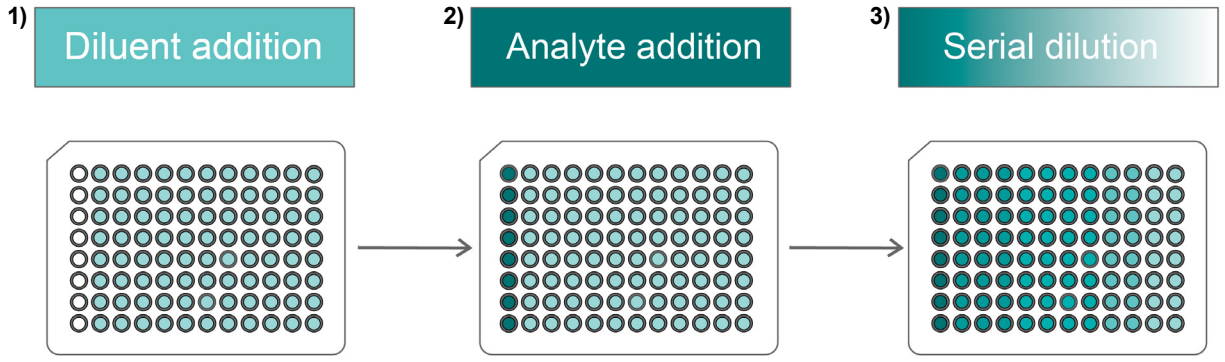

Figure 1: Experimental set-up for serial dilutions.

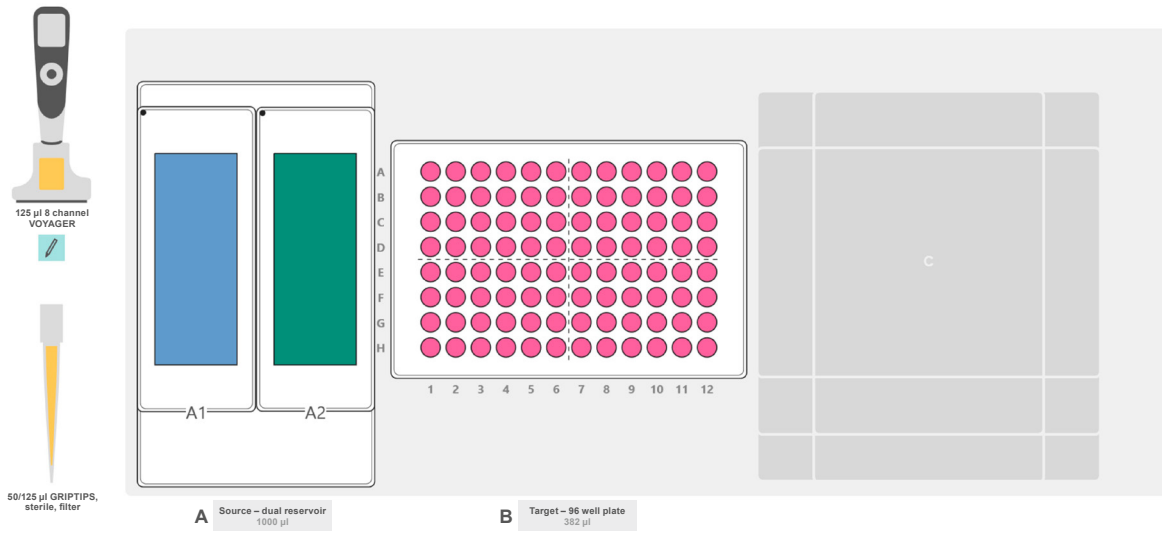

Figure 2: Deck set-up for performing serial dilutions. **Position A:** Source – dual reservoir adapter with 2x10 ml reservoirs; diluent in A1 (blue) and analyte in A2 (green). **Position B:** Target – 96 well flat bottom plate (pink). **Position C:** Empty.

Step-by-step procedure:

1. Serial dilution of an analyte

STEP: Serial dilution of an analyte.

HOW TO: The INTEGRA dual reservoir adapter, together with 2x10 ml reservoirs, is placed on deck Position A, with diluent (blue) in A1 and analyte (green) in A2 (**Figure 2**). A clear 96 well flat bottom plate (pink) is placed in landscape orientation on deck Position B (**Figure 2**).

Select and run one of the following VIALAB programs:

2-fold serial dilution → 125_VOYAGER_2_fold_serial_dilution
5-fold serial dilution → 125_VOYAGER_5_fold_serial_dilution
10-fold serial dilution → 125_VOYAGER_10_fold_serial_dilution

Specific volumes are handled by the VOYAGER (**Figure 3**). The diluent is transferred in multiple dispensing steps from the reservoir (Position A – A1) into each well of the 96 well flat bottom plate, starting with column 2 (Position B). To ensure precision during plate set-up, a pre- and post-dispense of 5 % of the transferred volume is used for the 10-fold serial dilution, and 10 % for the 2- and 5-fold serial dilutions.

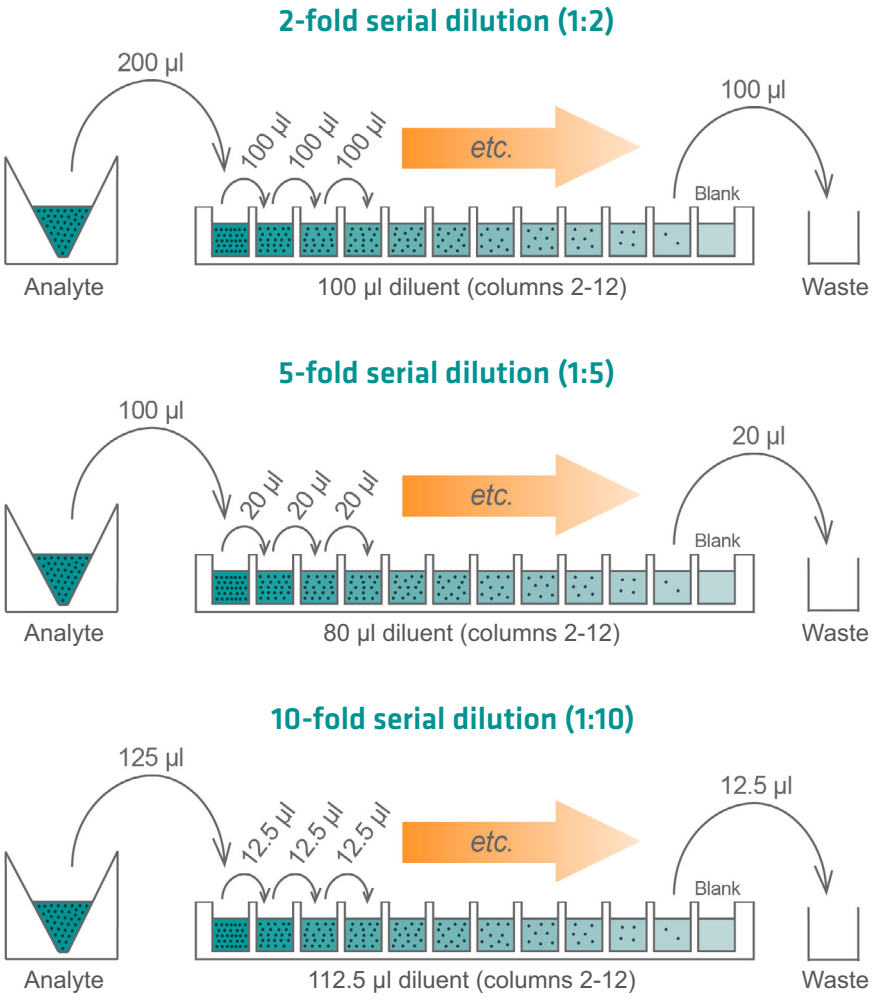

Figure 3: Serial dilutions in 96 well flat bottom plates with the 125 µl VOYAGER.

Using new GRIPTIPS, the VOYAGER aspirates the highest concentration of analyte from the reservoir (Position A – A2) and dispenses it into the first column of the 96 well flat bottom plate (Position B).

Without changing the GRIPTIPS, the VOYAGER begins the serial dilution by aspirating the specific volume (**Figure 3**) from column 1 of the 96 well flat bottom plate (Position B) and dispensing into the second column. The VOYAGER then mixes 100 µl (2- and 10-fold serial dilution) or 80 µl (5-fold serial dilutions) of the analyte/diluent 5 times at maximum speed (10). A blowout is performed to clear the tip of any remaining liquid before aspirating for the following dilution step. The procedure is repeated until column 11 is reached, where the last aspiration is discarded along with the GRIPTIPS (**Figure 4**). Column 12 only contains diluent, and functions as a blank for background noise elimination.

TIPS:

- Pre-wetting tips when pipetting aqueous liquids ensures excellent accuracy and precision.
- Using adjustable mixing cycles compensates for slower mixing speeds or poorly soluble analytes.

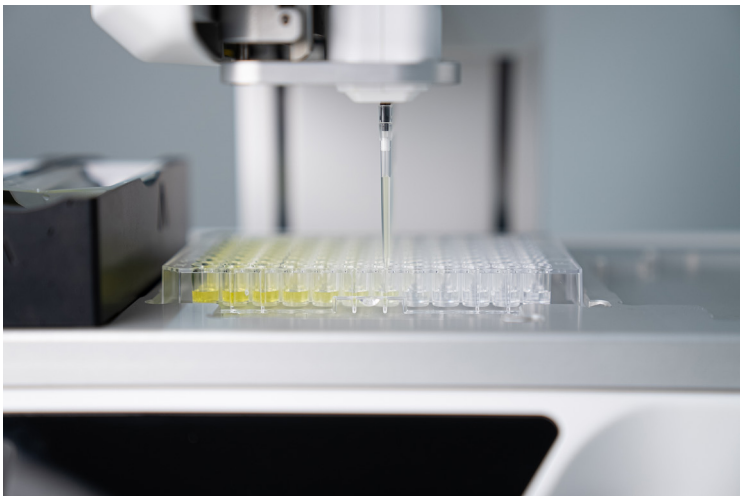

Figure 4: The ASSIST PLUS and VOYAGER performing serial dilution of tartrazine.

Results

The performance of the 8 channel 125 µl VOYAGER on the ASSIST PLUS during serial dilution of 0.36 mM tartrazine in water in 96 well flat bottom plates (**Figure 5**) was analyzed at 428 nm absorbance using the Tecan Infinite® M200 PRO. More detailed data is provided in INTEGRA's comprehensive guide to performing serial dilution.

Figure 5: 2-fold serial dilution of tartrazine in a 96 well flat bottom plate.

Figure 6 shows a representational, optimized calibration curve of a 2-fold serial dilution. Automating all liquid handling steps and mixing 100 µl of each dilution (>80 % GRIPTIPS volume) 5 times at maximum speed (10) led to reliable results in 3 independent runs. Furthermore, final values of less than 1 % were calculated for the accuracy and precision of the individual dilution steps.

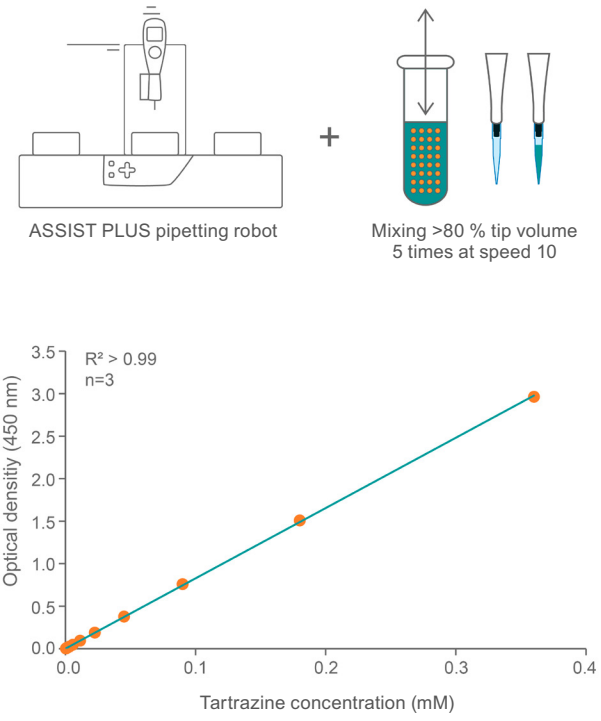

Figure 6: Result of a 2-fold serial dilution of tartrazine using optimized mixing settings on the ASSIST PLUS.

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Remarks

- **VIALAB software:** VIALAB programs can be easily adapted to your specific labware and protocols, such as when partial plates are needed.
- **Partial plates:** Programs can be adapted at any time to accommodate varying sample numbers, giving laboratories total flexibility to meet current and future demands.

Conclusion

- Automated workflows on the ASSIST PLUS offer reproducible results and eliminate any operator influence on serial dilutions.
- INTEGRA's electronic pipettes ensure homogeneity of aqueous solutions with dynamic mixing of each dilution. This is achieved by aspirating and dispensing >80 % of the total GRIPTIPS or reaction volume, repeated 5 times at speed 10.
- Understanding how to perform serial dilutions is crucial to optimize workflows and prevent error propagation. The automated protocols on the ASSIST PLUS provide optimal liquid handling settings for 2-, 5- and 10-fold serial dilutions.
- The ASSIST PLUS has a compact footprint to enable risk-free dilution of hazardous compounds in a biosafety cabinet.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4722	125 µl 8 channel VOYAGER electronic pipette	https://www.integra-biosciences.com/en/electronic-pipettes/voyager
INTEGRA Biosciences	4547	Dual reservoir adapter	https://www.integra-biosciences.com/en/pipetting-robots/assist-plus
INTEGRA Biosciences	4372	10 ml divided reservoir, polystyrene SureFlo™	https://www.integra-biosciences.com/en/reagent-reservoirs/multichannel-reagent-reservoirs
INTEGRA Biosciences	6465	125 µl sterile, filter GRIPTIPS	https://www.integra-biosciences.com/en/pipette-tips/grip-tip-selector-guide
Greiner Bio-One International	655161	96 well microplate, PS, F-bottom	https://shop.gbo.com/en/germany/products/bioscience/microplates/96-well-microplates/96-well-microplates-clear/655161.html

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