

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

## Introduction

Serial dilution, a reduction in concentration by constant dilution factor, is a common approach for screening related applications, such as the determination of minimum inhibitory concentrations (MIC) in drug discovery, the most probable numbers (MPN) in microbiology or general nucleic acid quantifications in molecular biology. Despite being a simple technique, poor liquid handling of interdependent dilution steps can cause error propagation and accumulation. Thorough mixing, therefore, is crucial but puts a lot of strain on the thumb, which increases the risk of repetitive strain

injuries. Furthermore, when done regularly, serial dilutions are significantly time-consuming procedures.

Here we show the simplest way of how to do serial dilutions with the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot to gain more walk-away time. The provided protocols detail optimal settings with reliable results when diluting analytes into water. In our comprehensive guide you can learn even more about key parameters when facing different conditions.

### Key benefits:

- Proven serial dilution protocols with optimal settings for the VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot guarantee uniform pipetting and mixing.
- INTEGRA's electronic pipettes prevent thumb overtaxing during liquid handling steps and, together with the ASSIST PLUS, enable risk-free handling of hazardous samples.
- Profit from additional hands-free time with the ASSIST PLUS and stop wasting time doing simple standard procedures manually.
- Game-changing liquid handling for 2-, 5- and 10-fold serial dilutions, with dynamic mixing volumes ensuring homogeneity of analytes.
- Simplified workflow with VIALAB's serial dilution protocol, including individual mixing parameters to cope with poorly soluble analytes.
- More flexibility during automated serial dilution with the VOYAGER when performing serial dilutions between different tubes and plates. Furthermore, the pipette can be easily changed to another volume size while using the same protocol.

## Overview: How to do serial dilution with the ASSIST PLUS pipetting robot

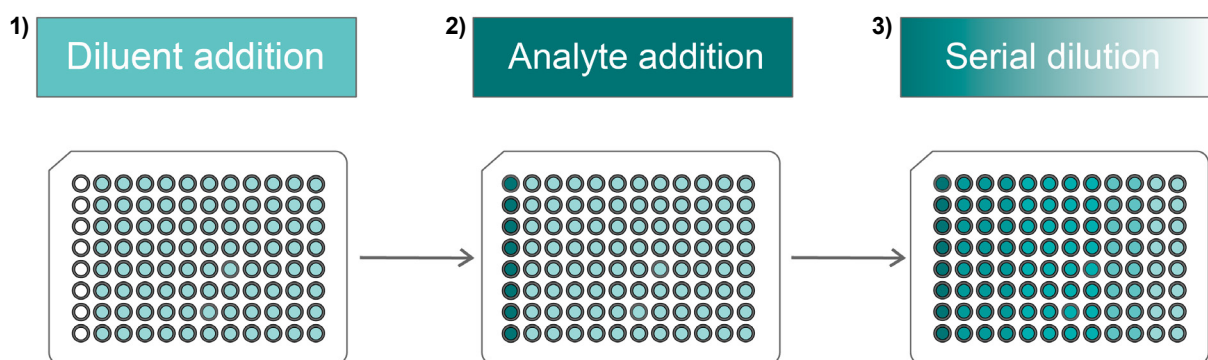


Here we show how to do serial dilution of tartrazine in water with an 8 channel 125 µl VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot.

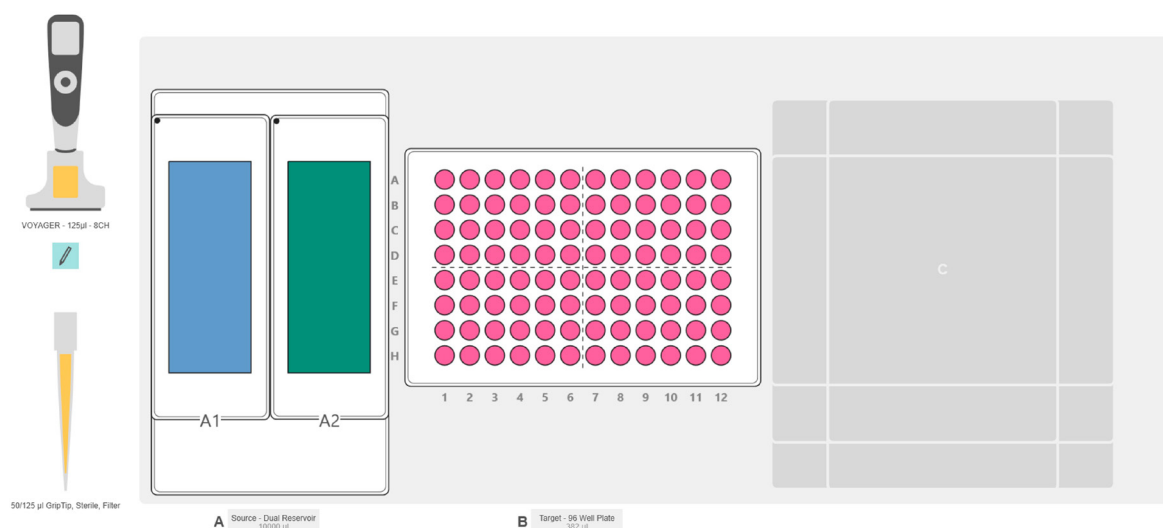
## Experimental set-up

The ASSIST PLUS pipetting robot, together with the 8 channel 125 µl VOYAGER adjustable tip spacing pipette and 125 µl sterile, filter GRIPTIPS®, automates complete serial dilutions in one program consisting of three 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:** How do serial dilution (experimental set-up).



**Figure 2:** Deck set-up for serial dilution. **Position A:** Source – dual reservoir adapter with two 10 ml reservoir; 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 two 10 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**).

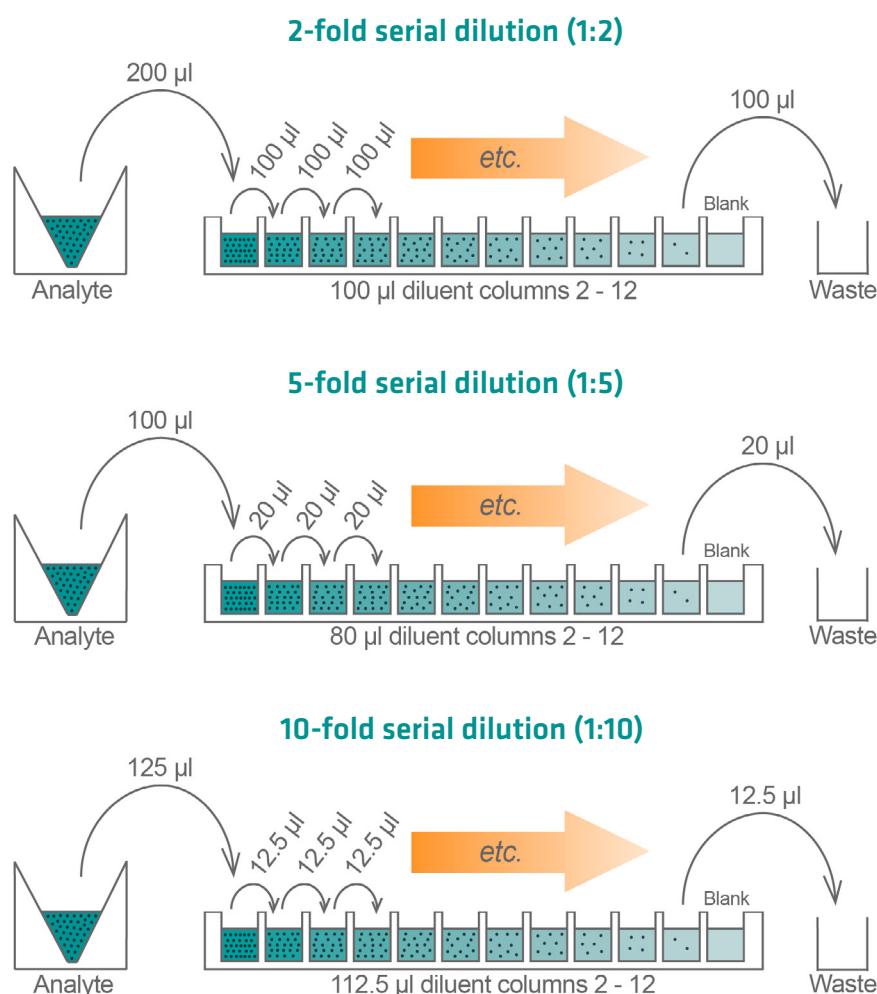
Select and run one of the following VIALAB programs:

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 adjustable tip spacing pipette (**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 two (Position B). For each dispense, a pre- and post-dispense with 5 % (10-fold serial dilutions) or 10 % (2- and 5-fold serial dilutions) of the transferred volume is used to guarantee precision during plate set-up.



**Figure 3:** How to do serial dilutions in 96 well flat-bottom plates with the 125 µl VOYAGER.

Using new GRIPTIPS<sup>®</sup>, 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<sup>®</sup>, the VOYAGER begins the serial dilution by aspirating the specific volume (**Figure 3**) from column one of the 96 well flat bottom plate (Position B) and dispensing into the second column. The VOYAGER mixes then 100  $\mu\text{l}$  (2- and 10-fold serial dilutions) or 80  $\mu\text{l}$  (5-fold serial dilutions) of the analyte/diluent five times at maximum speed (10). Before aspirating for the following dilution step, a blow out is performed to clear the tip of any remaining liquid. The procedure is repeated until column 11 is reached, where the last aspiration is discarded along with the GRIPTIPS<sup>®</sup> (**Figure 4**). Column 12 only contains diluent, and functions as blank for background noise elimination.

**TIPS:**

- Excellent accuracy and precision with tip pre-wetting when pipetting aqueous liquids.
- Adjustable mixing cycles to compensate slower mixing speed or poorly soluble analytes.



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

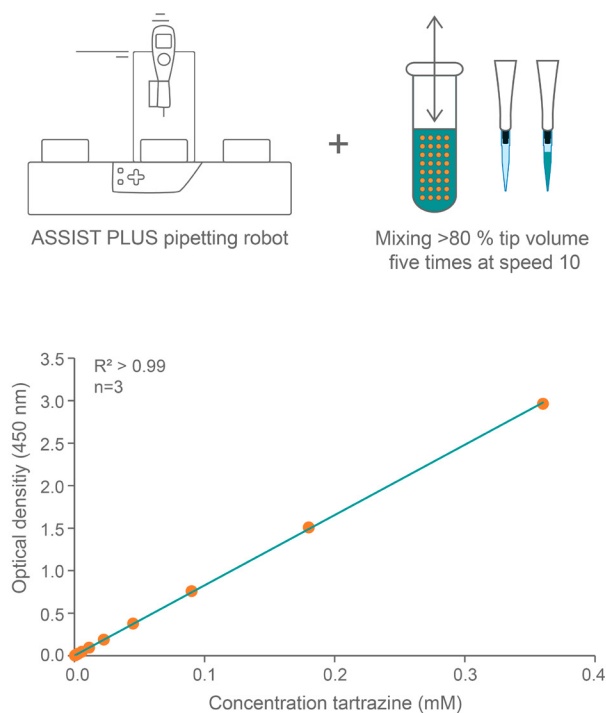
## Results

The performance of the 8 channel 125  $\mu\text{l}$  VOYAGER adjustable tip spacing pipette on the ASSIST PLUS pipetting robot during serial dilution of 0.36 mM tartrazine in water in 96 well flat bottom plates (**Figure 5**) was analyzed at 428 nm absorbance (Tecan Infinite<sup>®</sup> M200PRO). Detailed data is provided in our comprehensive guide on how to perform 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 each dilution with 100  $\mu\text{l}$  (>80 % GRIPTIPS<sup>®</sup> volume) five times at maximum speed (10), led to reliable results in three independent runs. Furthermore, final values of below 1 % were calculated for the accuracy and precision of the individual dilution steps.



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

## Remarks

- **VIALAB software:** VIALAB programs can be easily adapted to your specific pipette, labware and protocols, for instance when partial plates are needed.
- **Partial plates:** Programs can be adapted at any time to a different number of samples, giving laboratories total flexibility to meet current and future demands.

## Conclusion

- Profit from reproducible results and eliminate any operator influence on serial dilutions with automated workflows on the ASSIST PLUS pipetting robot.
- Guaranteed homogeneity of aqueous solutions with dynamic mixing of each dilution is only possible when using INTEGRA's electronic pipettes (80 % of total GRIPTIPS® or reaction volume, five times, speed 10).
- Understanding how to perform serial dilutions is crucial to optimizing workflows and preventing error propagation. The automated protocols on the ASSIST PLUS provide the best liquid handling settings for 2-fold serial dilutions, 5-fold serial dilutions and 10-fold serial dilutions.
- Risk-free dilution of hazardous compounds in a biosafety cabinet is possible thanks to the compact footprint of the ASSIST PLUS.

## Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	4505	ASSIST PLUS base unit	<a href="https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus">https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus</a>
INTEGRA Biosciences	4722	VOYAGER 8 channel 125 µl electronic pipette	<a href="https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager">https://www.integra-biosciences.com/global/en/electronic-pipettes/voyager</a>
INTEGRA Biosciences	4547	Dual Reservoir Adapter	<a href="https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus">https://www.integra-biosciences.com/global/en/pipetting-robots/assist-plus</a>
INTEGRA Biosciences	4372	10 ml Divided Reservoir, Polystyrene SureFlo™	<a href="https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs">https://www.integra-biosciences.com/global/en/reagent-reservoirs/multichannel-reagent-reservoirs</a>
INTEGRA Biosciences	6465	125 µl Sterile, Filter GRIPTIPS®	<a href="https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide">https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide</a>
Greiner Bio-One International	655161	96 Well Microplate, PS, F-Bottom	<a href="https://shop.gbo.com/en/germany/products/bioscience/microplates/96-well-microplates/96-well-microplates-clear/655161.html?_ga=2.194844971.761907635.1609937175-74237308.1609937175">https://shop.gbo.com/en/germany/products/bioscience/microplates/96-well-microplates/96-well-microplates-clear/655161.html?_ga=2.194844971.761907635.1609937175-74237308.1609937175</a>

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