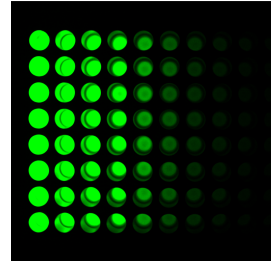


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Fluorescein Serial Dilution (1:2) 荧光素梯度稀释

DOI

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External link: <https://github.com/KRATSZ/LabScript-AI>



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Protocol status: Working

We use this protocol and it's working

Created: November 25, 2025

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Protocol Integer ID: 233530

Keywords: Opentrons, Serial Dilution, Fluorescence, Calibration, Python Protocol, Laboratory Automation, 96-well plate, PBS, Fluorescein, Liquid Handling, fluorescein serial dilution, serial dilution of fluorescein, calibrating plate reader fluorescence, plate reader fluorescence, serial dilution, fluorescein, dilution series, smart pipetting, opentrons flex robot

Disclaimer

This protocol is for research use only and is not intended for diagnostic procedures. The author assumes no responsibility for any damage to equipment or loss of samples resulting from the use of this code. Users should verify the robot calibration and labware definitions before running the protocol with valuable reagents.

Abstract

This protocol performs an automated 1:2 serial dilution of fluorescein across columns 1–11 of a 96-well plate using the Opentrons Flex robot. It is designed for calibrating plate reader fluorescence.

- **Flexibility:** Supports 1–8 parallel replicates.
- **Smart Pipetting:** Automatically selects between 1-channel (for <8 samples) and 8-channel pipettes (for 8 samples).
- **Outcome:** Columns 1–11 contain the dilution series; Column 12 serves as a PBS blank.

Attachments



荧光素梯度稀释.png
753KB



Photo-LayOut-荧光素梯度稀释...
3.6MB



荧光素梯度稀释标定.py
6KB

Image Attribution

All images and diagrams were created by the author.

Guidelines

- **Labware Definitions:** Ensure the labware placed on the deck exactly matches the definitions in the Python protocol (96_wellplate_360ul_flat and nest_12_reservoir_15ml). Using different labware dimensions may result in crashes or inaccurate pipetting.
- **Pipette Calibration:** Verify that the pipettes (Flex 1-channel and 8-channel) are calibrated and the deck calibration is up to date.
- **Dead Volume:** Always add slightly more reagent than calculated to account for the reservoir dead volume (recommend adding +500 μ L extra).
- **Tip Usage:** The protocol assumes a full tip rack at the start. If using a partially used rack, ensure columns 1, 2, and the necessary columns for dilution are present.

Materials

Robot: Flex

- **Pipettes:** Left `flex1channel1000` (8 replicates), Right `flex8channel1000` (=8 replicates)

Deck Layout

Position	Item	Role
---	---	---
B2	96-well plate (Dilution Plate)	Serial dilution
B3	12-well reagent reservoir	Reagents (stock, diluent)
C2	200 μ L tiprack	Tips
A3	Trash bin	Liquid waste

Reagents (Reservoir)

Well	Reagent	Details
---	---	---
A1	Fluorescein 1X stock	Source for dilution series
A2	PBS 1X	Diluent



Safety warnings

- ❗ **Light Sensitivity:** Fluorescein is light-sensitive. Prolonged exposure to bright light can affect fluorescence readings. Analyze the plate immediately after the protocol finishes.
- **Collision Risk:** Do not place any tall labware in unused slots (A1, B1, D1, D2) that might interfere with the gantry movement.
- **Liquid Waste:** The protocol discards liquid into the trash bin. Ensure the bin liner is not leaking.

Ethics statement

This protocol involves standard chemical reagents and does not involve human subjects or animal experimentation. No ethical approval is required.

Before start

1. **Power On:** Switch on the Opentrons Flex and connect via the Opentrons App.
2. **Trash Check:** Ensure the Trash Bin in Slot A3 is empty and properly installed.
3. **Tip Loading:** Place a full 200 μ L tip rack in Slot C2.
4. **Plate Loading:** Place a clean, empty Corning 96-well flat-bottom plate in Slot B2.
5. **Reagent Loading:** Fill the NEST 12-well reservoir in Slot B3:
 - **Well A1:** Add at least 2.5 mL of Fluorescein (1X Stock).
 - **Well A2:** Add at least 12 mL of PBS (Diluent).

Lighting: If possible, dim the room lights or cover the reservoir to prevent photobleaching of the fluorescein before the run starts.



Abstract

- 1 This protocol performs an automated 1:2 serial dilution of fluorescein across columns 1–11 of a 96-well plate using the Opentrons Flex robot. It is designed for calibrating plate reader fluorescence.
 - **Flexibility:** Supports 1–8 parallel replicates.
 - **Smart Pipetting:** Automatically selects between 1-channel (for <8 samples) and 8-channel pipettes (for 8 samples).
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Materials & Equipment

- 2 **Reagents:**
 - Fluorescein (1X Stock Solution)
 - PBS (Phosphate Buffered Saline, 1X)**Labware (Opentrons Definitions):**
 - Corning 96 Well Plate 360 µL Flat (corning_96_wellplate_360ul_flat)
 - NEST 12 Well Reservoir 15 mL (nest_12_reservoir_15ml)
 - Opentrons Flex 96 Tip Rack 200 µL (opentrons_flex_96_tiprack_200ul)**Equipment:**
 - Opentrons Flex Robot
 - Flex 1-Channel Pipette 1000 µL (Mount: Left)
 - Flex 8-Channel Pipette 1000 µL (Mount: Right)

Procedure

3 Section 1: Deck & Reagent Setup

Step 1: Deck Layout

Place the labware on the Opentrons Flex deck as defined below:

	Position	Item	Role
	B2	96-well Plate	Dilution Target
	B3	12-well Reservoir	Reagent Source
	C2	200 µL Tip Rack	Tips
	A3	Trash Bin	Waste

 Opentrons_FLEX_device2D平面图.png

equipment

 Photo-LayOut-荧光素梯度稀释.jpg

layout

4 **Step 2: Reagent Preparation (Reservoir B3)**

Fill the NEST 12-well reservoir (Slot B3) as follows:

- **Well A1:** Fluorescein 1X Stock (Min. 2 mL)
- **Well A2:** PBS 1X (Diluent) (Min. 10 mL)

5 **Step 3: Protocol Parameters**

Load the Python protocol. Set the parallel_samples parameter (Runtime Parameter):

- **1–7 samples:** Robot uses Left Mount (1-channel pipette).
- **8 samples:** Robot uses Right Mount (8-channel pipette).

6 **Step 4: Phase 1 – Plate Preparation (Automated)**

The robot will perform the following actions automatically:

Add Diluent: Dispense 100 µL PBS into Columns 2–12.

- Note: Uses new tips per column.

Add Sample: Dispense 200 µL Fluorescein Stock into Column 1.

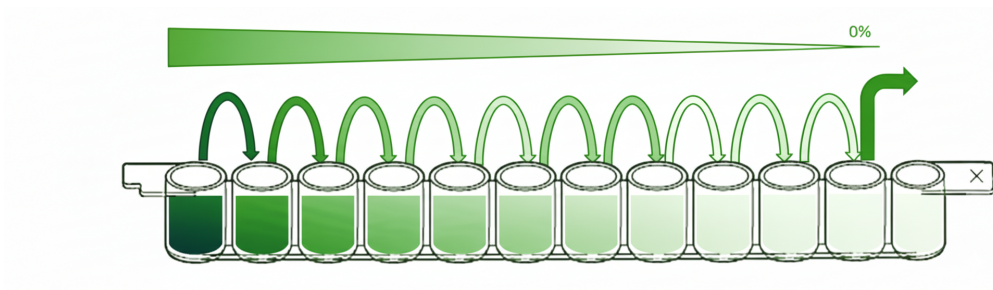
7 **Step 5: Phase 2 – Serial Dilution (Automated)**

The robot performs the 1:2 dilution series:

1. **Transfer:** Aspirate 100 µL from Col 1 → Dispense to Col 2.
2. **Mix & Propagate**
 - Mix 3 times (150 µL volume).
 - Transfer 100 µL to the next column.
 - Repeat until Column 11.

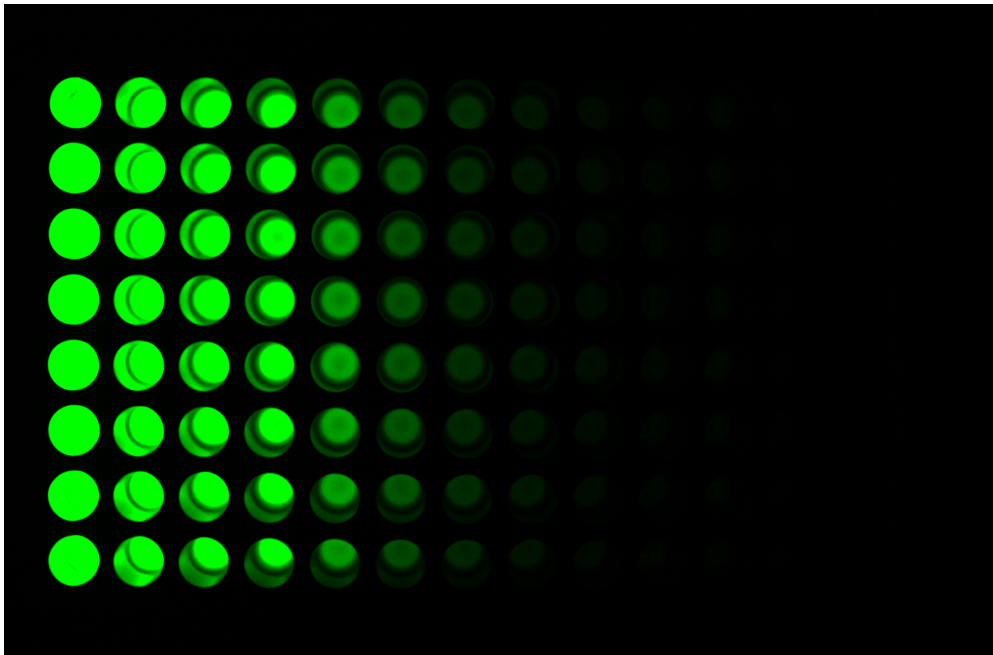
Final Step: After mixing Column 11, aspirate 100 µL and discard into Trash (A3).

- Result: Column 12 remains pure PBS (Blank).



Result

8



Warnings / Notes (注意事项)

- 9
- **Tip Usage:** Ensure the tip rack at C2 is full before starting. The protocol consumes 1 column of tips for PBS, 1 column for Fluorescein, and 1 column (or single tips) for the dilution series.
 - **Liquid Waste:** Ensure the Trash Bin is installed at A3.
 - **Calibration:** Ensure the robot deck calibration is up to date, especially for the 96-well plate, to avoid tip collision during mixing.

Protocol references

<https://www.protocols.io/view/calibration-protocol-plate-reader-fluorescence-cal-x54v986dpI3e/v3>