Cytochrome P450 Assay on the Agilent Bravo Platform

Application Bulletin

The Agilent Bravo Automated Liquid Handling Platform

Summary

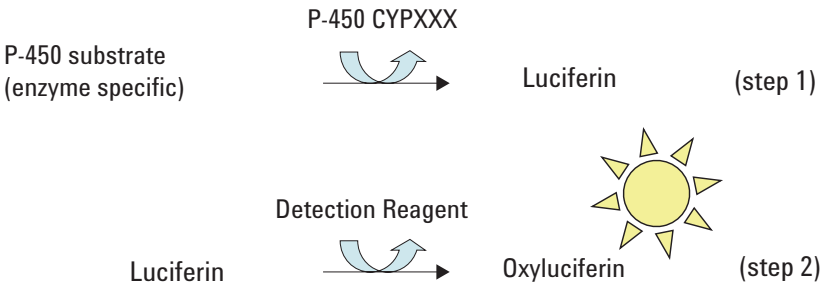
- Small footprint liquid handler provides 5 microplate throughput per run
- Microplate processing time is approximately 1 hour per 5 microplate run
- Throughput can be easily scaled with the addition of an Agilent BenchCel Microplate Handling Workstation

Introduction

Cytochrome P450 enzymes are the largest group of drug-metabolizing enzymes, and are therefore of critical importance in the drug discovery process. Their potential inhibition by drug candidates is an important part of ADME/Tox compound profiling. The demand for this type of assay is high because of the increasing output from high-throughput screening (HTS) and the pressure to frontload as much metabolic testing as possible during drug discovery. There are a number of different approaches for Cytochrome P450 testing. Here we outline a protocol for the Promega P450-Glo kit using the Agilent Bravo Platform. The Cytochrome P450 enzyme metabolizes a luminogenic substrate (step 1) into a luciferin product in the presence of an NADPH regenerating system. The addition of a luciferin detection reagent stops the enzyme reaction and converts the luciferin product into a luminescent signal (step 2), directly proportional to the enzyme activity.

System Description

The Promega P450-Glo kit is easily adapted for automation on a Bravo Platform that is equipped with a microplate gripper and a 384-channel pipette head. The microplate stacking feature allows multiple plates to be located at one deck position. The plates can quickly be re-stacked to allow for first in – first out processes. The reagents are added without tip touching, therefore only one tip box is needed per reagent. Efficient mixing is generated by the Orbital Shaking Station, which doubles as a pipetting position to optimize plate throughput. The plates are stacked on the Bravo Platform deck for room-temperature incubation. Agilent VWorks Automation Control software manages all process and incubation times, to guarantee reliable and repeatable results.



Overview of the two-step reaction involved in measuring Cytochrome P450 activity using a luminescent signal.

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Materials

Component List

- Agilent Bravo Platform with gripper, 384ST disposable-tip head
- Three reservoirs
- One Orbital Shaking Station
- Agilent VWorks Automation Control software

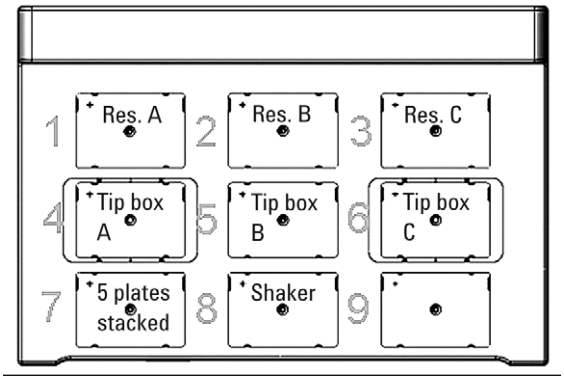
Labware List

- Microplate A (1-5): Greiner 384PS, white, flat-bottom
- Tipbox A, B, C: Agilent Tips 384 ST 70 µL

Reagent List

- Reservoir A: Cyp P450/Substrate mix
- Reservoir B: NADPH regenerating system solution
- Reservoir C: Luciferin detection reagent

Instrument Layout



The Agilent Bravo deck layout with one Orbital Shaking Station (location 8). One stack of five microplates (location 5), three reservoirs (locations 1-3), and three tipboxes (locations 4-6) are placed manually on the deck before the protocol is started.

Protocol Workflow

1. Attach tips at location 4.
2. Move microplate A-1 from location 7 to 8 (Shaker).
3. Dispense 6.25 µL P450/Substrate from reservoir A to microplate A-1.
4. Shake for 20 s.
5. Move microplate A-1 from location 8 to 9.
6. Incubate microplate A-1 for 10 min.

Loop: Repeat step 2 to 6 for microplates A-2 to A-5.

7. Remove tips at location 4.
8. Restack microplates A-1 through A-5 from location 9 to 7.
9. Press on tips at location 5.
10. Move microplate A-1 from location 7 to 8 (Shaker).
11. Dispense 12.5 µL NADPH from reservoir B to microplate A-1.
12. Shake for 20 s.
13. Move microplate A-1 from location 8 to 9.
14. Incubate microplate A-1 for 10 min.

Loop: Repeat steps 10 to 14 for microplates A-2 to A-5.

15. Remove tips at location 5.
16. Restack microplates A-1 to A-5 from location 9 to 7.
17. Press on tips at location 6.
18. Move microplate A-1 from location 7 to 8 (Shaker).
19. Dispense 25 µL Luciferin from reservoir C to microplate A-1.
20. Shake for 10 s.
21. Move microplate A-1 from location 8 to 9.
22. Incubate microplate A-1 for 20 min.

Loop: Repeat step 18 to 22 for microplates A-2 to A-5.

23. Remove tips at location 5.
24. Restack microplates A-1 to A-5 from 9 to 7.

Conclusions

The platform provides a reliable and efficient solution for quantifying Cytochrome P450 activity. Utilizing the plate stacking capabilities of the Bravo Platform, up to five plates can be run without user intervention. The typical throughput for this setup is about 1 hour for five plates, depending on exact protocol and liquid handling steps. This instrument can easily scale to meet increasing capacity demands with the integration of an Agilent BenchCel Microplate Handling Workstation.

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