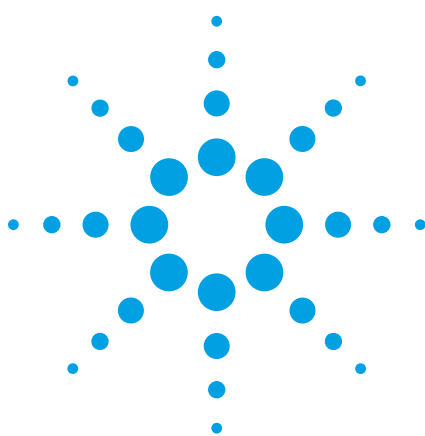


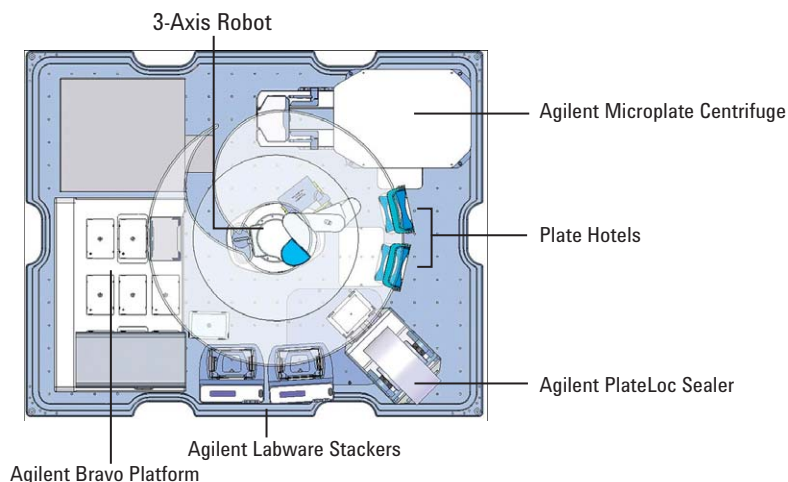
Cytochrome P450 Assay on the Agilent BioCel 900 System

Application Bulletin



Summary

- Small footprint automation system provides capacity for 20 microplates, without manual intervention
- Microplate processing time about x hour for a 20 plate run
- Efficient scheduling maximizes throughput and ensures same treatment for each plate



The Agilent BioCel 900 Automation Platform

Introduction

Cytochrome P450 (CYP450) enzymes are the largest group of drug-metabolizing enzymes and therefore critical targets for drug discovery. Inhibiting these enzymes 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 testing as possible during drug discovery. There are a number of different approaches for CYP450 testing. In this assay, the CYP450 enzyme metabolizes a fluorogenic substrate (step 1) into a highly fluorescent product in the presence of an NADPH regenerating system. The fluorescent signal (step 2) is directly proportional to the enzyme activity. Addition of CYP450 inhibitors results in a reduction in enzyme activity.

System Description

The Invitrogen Vivid kit is easily adapted for automation on the Agilent BioCel System. All liquid handling steps are carried out by the Agilent Bravo Automated Liquid Handling Platform. Pipetting can be performed without tip touching, preventing cross-contamination. Efficient mixing is generated by the Orbital Shaking Station, directly after reagent pipetting. The Plate Hotels store the assay plates during the run and for the room-temperature incubation step. Sample plates reside in Agilent Labware Stackers before and after processing. Agilent VWorks Automation Control software manages all processes and incubation times, to ensure reliable and repeatable results. This application bulletin outlines a protocol for the Invitrogen Vivid CYP450 Screening kit using the BioCel 900 System.

Materials

Component List

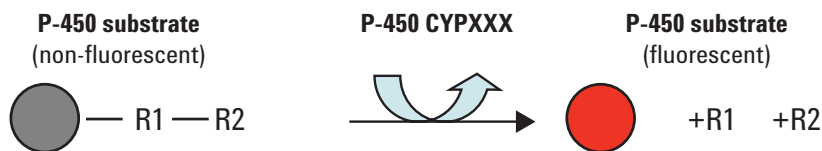
- Bravo Platform with 384ST disposable-tip head, 3 Reservoirs, 1 Tip Wash Station, 1 Orbital Shaking Station
- Two Labware Stackers
- Two Plate Hotels
- Agilent PlateLoc Thermal Microplate Sealer
- Agilent Microplate Centrifuge
- Tecan Spectrafluor plus

Labware List

- Microplate A (Assay Plate): Greiner 384PS, black, flat-bottom
- Microplate B (Sample Plate): Greiner 384PP, v-bottom
- Tipbox A, B, C, D: Agilent Tips 384 ST 70 μ L

Reagent List

- Reservoir A: Cyp P450/NADPH regenerating mix
- Reservoir B: Substrate/NADP+ mix
- Reservoir C: Stop reagent

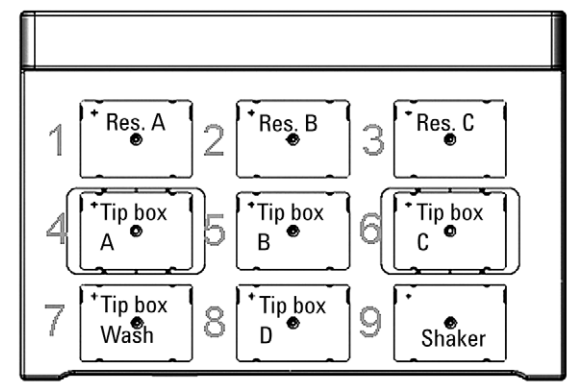


Overview of the metabolism of the fluorogenic "blocked" dye into a Fluorescent Metabolite (as outlined in: Invitrogen: Lit #Lo5o4 Rev. 06003)

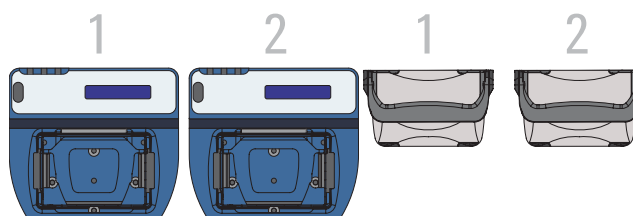


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Instrument Layout



The Agilent Bravo deck (top view). One Orbital Shaking Station (location 9), four reservoirs (locations 1-3, 7), and four tipboxes (locations 4-6, 8) are placed manually on the deck before the protocol is started.



Agilent Labware Stackers and Hotels (top view). Sample plates are stored in two Labware Stackers (left), with a capacity of 60 plates per Labware Stacker. Assay plates are stored and incubated in two Plate Hotels (right), with a capacity of 10 plates per Plate Hotel.

Protocol Workflow

1. Move microplate B from Labware Stacker 1 to Microplate Centrifuge.
2. Spin for 20 s.
3. Move microplate B from Microplate Centrifuge to Bravo location 9.
4. Press on tips at location 8.
5. Aspirate 20 μ L of sample from microplate B.
6. Move microplate B from Bravo location 9 to the PlateLoc.
7. Seal microplate B.
8. Move microplate B to Labware Stacker 2.
9. Move microplate A from Plate Hotel 1 to Bravo location 9.
10. Dispense 20 μ L of sample into microplate A.
11. Wash tips at location 7.
12. Release tips at location 8.
13. Press on tips at location 4.
14. Aspirate 20 μ L Cyp450 mix from reservoir A and dispense into microplate A.
15. Shake 20 s.
16. Release tips at location 4.
17. Move microplate A from Bravo location 9 to Plate Hotel 1.
18. Incubate for 20 min.
19. Move microplate A from Plate Hotel 1 to Bravo location 9.
20. Press on tips at location 5.
21. Aspirate 25 μ L Substrate mix from reservoir B and dispense into microplate A.
22. Shake 20 s.
23. Release tips at location 5.
24. Move microplate A from Bravo location 9 to the Spectrafluor device.
25. Read microplate A.
26. Move microplate A from the Spectrafluor device to Plate Hotel 1.
27. Incubate 15 min.
28. Move microplate from Plate Hotel 1 to Bravo location 9.
29. Press on tips at location 6.
30. Aspirate 25 μ L Stop mix from reservoir C and dispense into microplate A.
31. Shake 20 s.
32. Release tips at location 6.
33. Move microplate A from location 9 to the Spectrafluor device.
34. Read microplate A.
35. Move microplate A from the Spectrafluor device to Plate Hotel 1.

Conclusions

The Agilent BioCel 900 System provides a robust, high-throughput solution for quantifying Cytochrome P450 activity. The small footprint and open-access of the BioCel System makes it an ideal solution for medium to high throughput ADME/Tox applications, such as the CYP450 assay outlined here. All microplates are handled and processed in the same manner, including constant incubation times, ensuring reliable and repeatable results.

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