



Rat P450 cDNA Plate Array

Catalog Number AP-1181

(For Research Use Only)

Introduction

The cytochrome P450s are a multiple gene family of enzymes, playing central roles in oxidative metabolism of a wide range of xenobiotics, including chemicals, sterols, fatty acids and anticancer drugs. Many of P450s can activate cancer carcinogen, which contribute to tumorigenesis. They are highly differentially expressed in response to a variety of environmental chemicals and pharmaceuticals. As rat is often chosen as an animal model for the study of toxicity, profile of toxicity-related p450 expression is critical to uncovering the underlying mechanism. Signosis developed Rat P450 cDNA Plate Array for profiling the expression of a panel of 22 CYP genes.

Principle of the assay

Signosis' proprietary cDNA plate array is a plate-based hybridization profiling analysis for monitoring the expression of dozens of genes through reverse transcription of mRNA into cDNA. Like array analyses, total RNA is first reverse transcribed into cDNA in the presence of biotin-dUTP in the assay. Targeted genes are then specifically captured onto individual wells on a plate, instead of membranes, through a pre-coated gene-specific oligonucleotide. The captured cDNAs are further detected with streptavidin-HRP. Luminescence is reported as relative light units (RLUs) on a microplate luminometer. The expression level of genes is directly proportional to the luminescent intensity.

Materials provided with the kit

- A 96-well plate coated with 22 different capture oligos (RT)
- Rat P450 Primer Mix (-20 °C)
- Reverse transcription buffer mix (-20 °C)
- Reverse transcriptase RT (-20 °C)
- Streptavidin-HRP conjugate (4°C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)

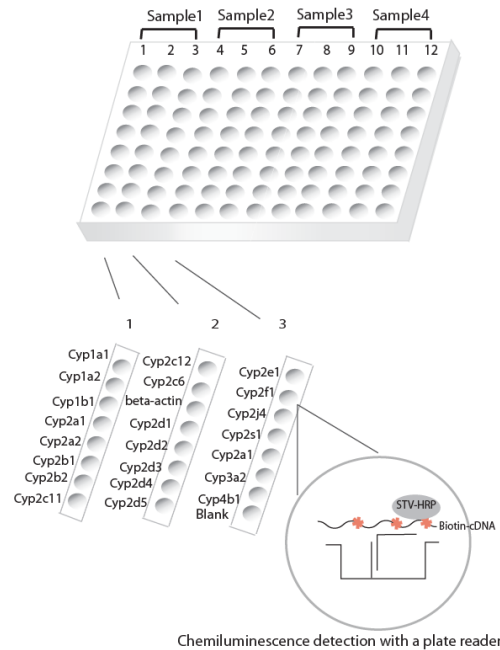


Diagram of Rat P450 cDNA plate array

Material required but not provided

- PCR machine
- Incubator
- 0.2ml PCR tube
- Luminometer plate reader
- ddH₂O (RNAase free)

Reagent preparation before starting experiment

- Dilute 30ml of 5x Plate hybridization wash buffer with 120 ml of dH₂O before use.
- Dilute 40ml of 5x Detection Wash Buffer with 160 ml of dH₂O before use.
- Warm up Plate hybridization buffer and Hybridization Wash buffer at 45 °C until no visible precipitate before use. Stir the solution with 10ml or 5ml pipette to facilitate the dissolving process.
- Dilute 500 times of streptavidin-HRP with blocking buffer before use at Step 3(4).

Assay procedure

1. cDNA synthesis

Note: Briefly spin tubes before opening

- (1) Sample preparation
 - X µl 1-10µg total RNA
 - 2 µl Rat P450 Primer Mix
 - X µl ddH₂O

- (2) Incubate for 5 minutes at 65 °C, and chill on ice.
- (3) Add 8 µl Reverse transcription buffer mix and 1µl RT to each reaction tube, and incubate for 1 hour at 45 °C.
- (4) Heat the reaction to 98 °C for 5 minutes, and chill on ice.
- (5) The 20ul cDNA is synthesized and labeled with biotin and ready for hybridization on the plate.

2. Plate hybridization

- (1) Remove the sealing film
- (2) Arrange the appropriate number of the wells of the plate based on your experiment. The whole plate is divided into 4 repeat sections, Column 1-3, 4-6, 7-9, 10-12 for 4 different samples.
- (3) Mix 20ul cDNA with 2.8ml pre-warmed Plate hybridization buffer, and dispense 95ul mixture to each well in a section **immediately**. A reagent reservoir can be used for dispensing cDNA mixture into the wells with a 8 multichannel pipette. Add 100ul Plate hybridization buffer without cDNA to the 'blank' well.
- (4) Seal the whole plate with foil film (provided) securely and incubate the plate at 45 °C for overnight. Ensure the numbers and letters on the plate are clearly visible from under foil seal by pressing the foil down on every single experimental well. *Put an open container with water in the incubator to keep humidity and prevent evaporation from experimental wells.*

3. Plate detection

- (1) Removing the top foil sealing film with a blade to expose the experimental wells. Keep the unused well sealed for the future usage.
- (2) Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate by adding 300µl of warmed 1x Plate hybridization wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.

- (3) Add 200µl of Blocking buffer incubate for 15 minutes at room temperature with gentle shaking.
- (4) Invert the plate over an appropriate container to remove blocking buffer. And add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
- (5) Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate with 200ul 1X Detection wash buffer for 5 min at room temperature with gently shaking. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- (6) Repeat (5) for additional 2 time washes.
- (7) Freshly prepare the substrate solution
For the whole plate:
1ml Substrate A
1ml Substrate B
8ml Substrate dilution buffer
- (8) Add 95µl substrate solution to each well and incubate for 1minutes.
- (9) Place the plate in the luminometer, and read. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-20 minutes.

Example of Analysis Data

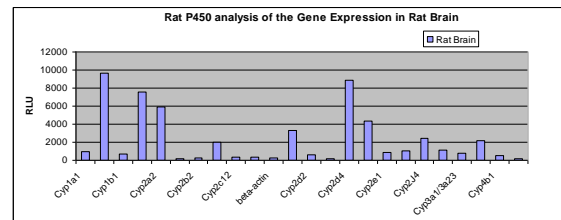


Figure1. Analysis of expression of P450 genes in rat brain with P450 cDNA Plate Array.

2ug of RNAs from rat brain was subjected to P450 cDNA plate assay. The cDNA plate array were detected with HRP by a luminometer plate reader.

Diagram of rat P450 cDNA plate array

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cyp1a1	Cyp2c12	Cyp2e1	Cyp1a1	Cyp2c12	Cyp2e1	Cyp1a1	Cyp2c12	Cyp2e1	Cyp1a1	Cyp2c12	Cyp2e1
B	Cyp1a2	Cyp2c6	Cyp2f1	Cyp1a2	Cyp2c6	Cyp2f1	Cyp1a2	Cyp2c6	Cyp2f1	Cyp1a2	Cyp2c6	Cyp2f1
C	Cyp1b1	beta-actin	Cyp2J4	Cyp1b1	beta-actin	Cyp2J4	Cyp1b1	beta-actin	Cyp2J4	Cyp1b1	beta-actin	Cyp2J4
D	Cyp2a1	Cyp2d1	Cyp2s1	Cyp2a1	Cyp2d1	Cyp2s1	Cyp2a1	Cyp2d1	Cyp2s1	Cyp2a1	Cyp2d1	Cyp2s1
E	Cyp2a2	Cyp2d2	Cyp3a1/3a23	Cyp2a2	Cyp2d2	Cyp3a1/3a23	Cyp2a2	Cyp2d2	Cyp3a1/3a23	Cyp2a2	Cyp2d2	Cyp3a1/3a23
F	Cyp2b1	Cyp2d3	Cyp3a2	Cyp2b1	Cyp2d3	Cyp3a2	Cyp2b1	Cyp2d3	Cyp3a2	Cyp2b1	Cyp2d3	Cyp3a2
G	Cyp2b2	Cyp2d4	Cyp4b1	Cyp2b2	Cyp2d4	Cyp4b1	Cyp2b2	Cyp2d4	Cyp4b1	Cyp2b2	Cyp2d4	Cyp4b1
H	Cyp2c11	Cyp2d5	Blank	Cyp2c11	Cyp2d5	Blank	Cyp2c11	Cyp2d5	Blank	Cyp2c11	Cyp2d5	Blank

