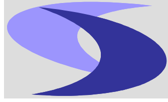

Signosis
Innovative Plate Assay Solutions

Human Toxicity cDNA Plate Array

Catalog Number AP-0203 **(For Research Use Only)**

Introduction

Drug toxicity is mainly resulted from drug metabolism. A drug may be biotransformed by drug metabolizing enzymes to toxic or nontoxic metabolites. A number of families such as P450s, UDP-glucuronosyltransferases, sulfotransferases, organic-anion transporters and multidrug resistance proteins involved in drug metabolizing processes, which catalyze the oxidation of exogenously administered drugs. The changes in expression level of these enzymes are the key factors responsible for the individual variation in drug metabolism. Signosis developed toxicity cDNA plate array to analyze the expression of 22 drug metabolism-related genes.

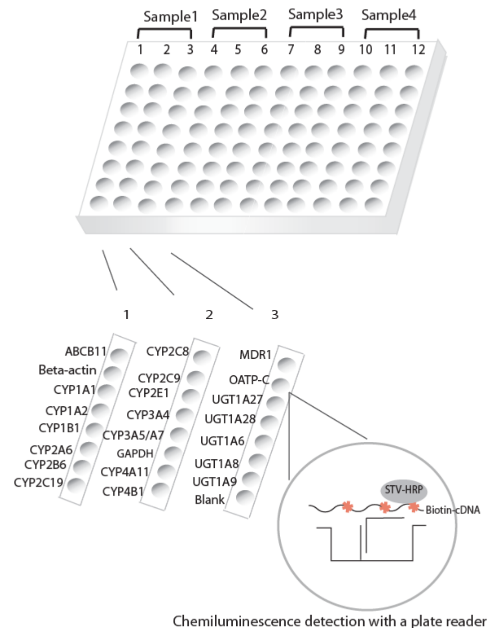

Diagram of human toxicity cDNA plate array

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)
- Human Toxicity Primer Mix (-20 ^{o}C)
- Reverse transcription buffer mix (-20 ^{o}C)
- Reverse transcriptase RT (-20 ^{o}C)
- Streptavidin-HRP conjugate (4°C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (RT)
- 5xDetection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (4°C)

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 dH_2O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH_2O before use.
- Warm up Plate hybridization buffer and Hybridization Wash buffer at 45 ^{o}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.

Signosis, Inc. • 1700 Wyatt Drive Suite 10-12 • Santa Clara, CA 95054 • Tel 408 747 0771 • Fax 408 864 2182

Assay procedure

1. cDNA synthesis using PCR machine
Note: Briefly spin tubes before opening

(1) Sample preparation

X µl 1-10µg total RNA
2 µl Human Toxicity Primer Mix
X µl ddH2O

11µl

(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.

3. Plate detection

(1) Remove the foil film from the experimental wells with a blade. Keep the unused well sealed.
(2) Invert the Hybridization Plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200ul of pre-warmed 1x Plate hybridization wash buffer to each well. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.
(3) Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
(4) Add 200µl of Blocking buffer to each well and incubate for 15 minutes at room temperature with gently shaking.
(5) Invert the plate over an appropriate container to remove block buffer.
(6) Add 20 µl of streptavidin-HRP conjugate in 10ml blocking buffer (1:500) dilution, enough for one plate. Add 95 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gently shaking.
(7) Wash the plate 3 times by adding 200ul 1X Detection wash buffer to each well, At each wash, incubate the plate for 10 minutes with gently shaking at room temperature.
(8) Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels. At the last wash, invert plate on clean paper towels for 1-2 min to remove excessive liquid.
(9) Freshly prepare the substrate solution:

For the whole plate:
1ml Substrate A
1ml Substrate B
8ml Substrate dilution buffer

(10) Add 95µl substrate solution to each well and incubate for 1 min.
(11) Place the plate in the luminometer. Allow plate to sit inside machine for 5 min before reading. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-20 minutes.

Diagram of human toxicity cDNA plate array

	1	2	3	4	5	6	7	8	9	10	11	12
A	ABCB11	CYP2C8	MDR1	ABCB11	CYP2C8	MDR1	ABCB11	CYP2C8	MDR1	ABCB11	CYP2C8	MDR1
B	Beta-Actin	CYP2C9	OATP-C	Beta-Actin	CYP2C9	OATP-C	Beta-Actin	CYP2C9	OATP-C	Beta-Actin	CYP2C9	OATP-C
C	CYP-1a1	CYP2E1	UGT1A27	CYP-1a1	CYP2E1	UGT1A27	CYP-1a1	CYP2E1	UGT1A27	CYP-1a1	CYP2E1	UGT1A27
D	CYP-1a2	CYP3A4	UGT1A28	CYP-1a2	CYP3A4	UGT1A28	CYP-1a2	CYP3A4	UGT1A28	CYP-1a2	CYP3A4	UGT1A28
E	CYP1B1	CYP3A5/A7	UGT1A6	CYP1B1	CYP3A5/A7	UGT1A6	CYP1B1	CYP3A5/A7	UGT1A6	CYP1B1	CYP3A5/A7	UGT1A6
F	CYP-2A6	GAPDH	UGT1A8	CYP-2A6	GAPDH	UGT1A8	CYP-2A6	GAPDH	UGT1A8	CYP-2A6	GAPDH	UGT1A8
G	CYP-2B6	CYP4A11	UGT1A9	CYP-2B6	CYP4A11	UGT1A9	CYP-2B6	CYP4A11	UGT1A9	CYP-2B6	CYP4A11	UGT1A9
H	CYP2C19	CYP4B1	Blank	CYP2C19	CYP4B1	Blank	CYP2C19	CYP4B1	Blank	CYP2C19	CYP4B1	Blank

	1	2	3	4	5	6	7	8	9	10	11	12
A	CYP1A1	CYP2E1	CYP11B1	CYP1A1	CYP2E1	CYP11B1	CYP1A1	CYP2E1	CYP11B1	CYP1A1	CYP2E1	CYP11B1
B	CYP1A2	CYP3A4	CYP17A1	CYP1A2	CYP3A4	CYP17A1	CYP1A2	CYP3A4	CYP17A1	CYP1A2	CYP3A4	CYP17A1
C	CYP1B1	CYP3A5/A7	CYP24A1	CYP1B1	CYP3A5/A	CYP24A1	CYP1B1	CYP3A5/A	CYP24A1	CYP1B1	CYP3A5/A	CYP24A1
D	Beta-actin	GAPDH	CYP26A1	Beta-actin	GAPDH	CYP26A1	Beta-actin	GAPDH	CYP26A1	Beta-actin	GAPDH	CYP26A1
E	CYP2A6	CYP4A11	CYP26B1	CYP2A6	CYP4A11	CYP26B1	CYP2A6	CYP4A11	CYP26B1	CYP2A6	CYP4A11	CYP26B1
F	CYP2B6	CYP4B1	CYP27A1	CYP2B6	CYP4B1	CYP27A1	CYP2B6	CYP4B1	CYP27A1	CYP2B6	CYP4B1	CYP27A1
G	CYP2C19	CYP4F2/F3/F8	CYP39A1	CYP2C19	CYP4F2/F3	CYP39A1	CYP2C19	CYP4F2/F3	CYP39A1	CYP2C19	CYP4F2/F3	CYP39A1
H	CYP2D6	CYP11A1	CYP46A1	CYP2D6	CYP11A1	CYP46A1	CYP2D6	CYP11A1	CYP46A1	CYP2D6	CYP11A1	CYP46A1