



Human Wnt/b-Catenin Regulated cDNA Plate Array

Catalog Number AP-0171

(For Research Use Only)

Introduction

The Wnt signaling pathway is important to both embryonic development and tumorigenesis. β -Catenin, the central component of the pathway, functions as a cofactor of the TCF/LEF family of transcription factors and activates transcription of Wnt target genes involved in cell proliferation, survival, and migration. A number of proteins including the tumor suppressor APC and Axin are involved in the regulation of the Wnt signaling pathway. Signosis' Wnt/ β -Catenin signaling pathway cDNA plate array allows measuring the expression of 21 genes that participate in the pathway at a same time. The difference of these genes in expression among different samples can be determined and compared by using the assay kit. Signosis' Wnt/ β -Catenin cDNA Plate Array allows profiling 20+ related 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 23 different capture oligos (RT)
- Human Wnt Reg.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)
- 5xDetection wash buffer (RT)
- Substrate A (4 °C)
- Substrate B (4 °C)
- Substrate dilution buffer (RT)

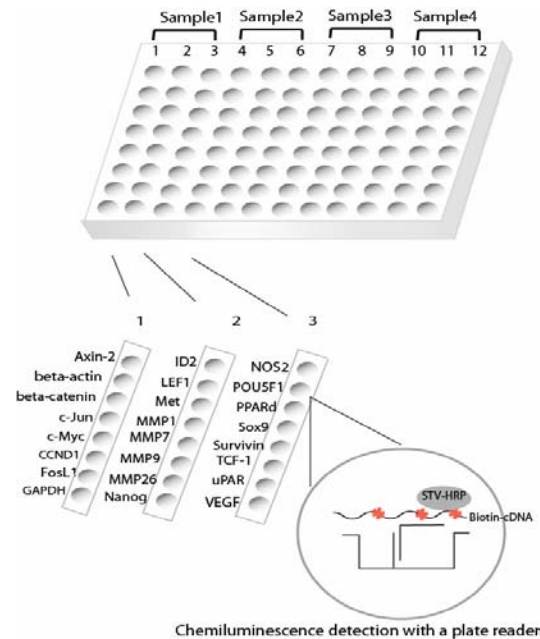


Diagram of human Wnt/ β -Catenin regulated cDNA plate array

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 using PCR machine

Note: Briefly spin tubes before opening

- (1) Sample preparation
 - X µl 1-10µg total RNA
 - 2 µl Human NFκB Reg. 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.
- (4) Seal the wells with foil film securely and hybridize 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 Data Analysis

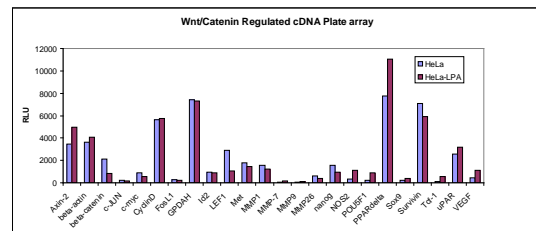


Figure1: Human Wnt/Catenin regulated cDNA plate assay for analysis of 21 genes.
HeLa cells were treated with and without 2 uM LPA (lysophosphatidic acid) for 8 hours, RNAs prepared, cDNA synthesized with biotin label and subjected to cDNA plate array hybridization and detection.

Diagram of Human Wnt/Catenin-regulated cDNA plate array

	1	2	3	4	5	6	7	8	9	10	11	12
A	Axin-2	Id2	NOS2	Axin-2	Id2	NOS2	Axin-2	Id2	NOS2	Axin-2	Id2	NOS2
B	beta-actin	LEF1	POU5F1	beta-actin	LEF1	POU5F1	beta-actin	LEF1	POU5F1	beta-actin	LEF1	POU5F1
C	beta-caten	Met	PPARd	beta-caten	Met	PPARd	beta-caten	Met	PPARd	beta-caten	Met	PPARd
D	c-JUN	MMP1	Sox9	c-JUN	MMP1	Sox9	c-JUN	MMP1	Sox9	c-JUN	MMP1	Sox9
E	c-myc	MMP-7	Survivin	c-myc	MMP-7	Survivin	c-myc	MMP-7	Survivin	c-myc	MMP-7	Survivin
F	CyclinD	MMP9	Tcf-1	CyclinD	MMP9	Tcf-1	CyclinD	MMP9	Tcf-1	CyclinD	MMP9	Tcf-1
G	FosL1	MMP26	uPAR	FosL1	MMP26	uPAR	FosL1	MMP26	uPAR	FosL1	MMP26	uPAR
H	GPDAH	nanog	VEGF	GPDAH	nanog	VEGF	GPDAH	nanog	VEGF	GPDAH	nanog	VEGF