



Mouse HIF-Regulated cDNA Plate Array

Catalog Number AP-1111

(For Research Use Only)

Introduction

Rapidly growing tumors result in hypoxic regions. Adaptive responses of most cells to hypoxia are (1) to produce VEGF and other hypoxia-induced angiogenic cytokines that promote increased tissue vascularization, thereby increasing tissue oxygenation, and (2) to switch metabolically from oxidative phosphorylation to anaerobic glycolysis. Hypoxia-inducible factor 1 (HIF-1) is an oxygen-regulated transcriptional activator that plays essential roles in the process. The HIF-1 alpha subunit is oxygen-dependent ubiquitination and proteasomal degradation. In addition, cytokines including interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) stimulate HIF-1 dependent gene expression. HIF-1 increases the expression of several genes that promote blood flow and inflammation, such as vascular endothelial growth factor (VEGF). Signosis' HIF 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)
- Mouse HIF 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 (4°C)

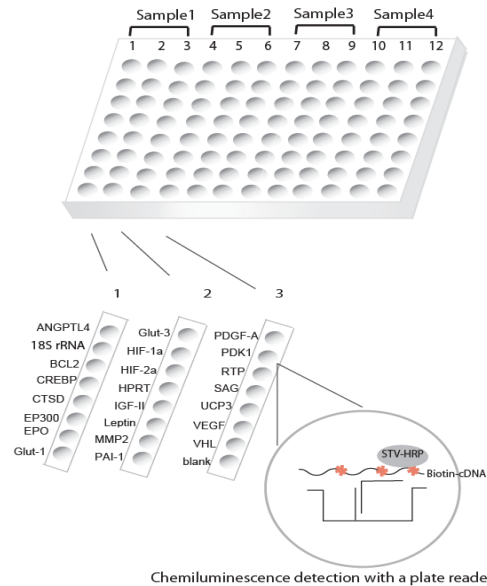


Diagram of Mouse HIF-regulated cDNA plate array

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.

Assay procedure

1. cDNA synthesis

Note: Briefly spin tubes before opening

- (1) Sample preparation
X μ l 1-10 μ g total RNA
2 μ l Mouse HIF Reg. Primer Mix
X μ l ddH₂O

11 μ l
- (2) Incubate for 5 minutes at 65 °C, and chill on ice.
- (3) Add 8 μ l Reverse transcription buffer mix and 1 μ l 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 20 μ l 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 20 μ l cDNA with 2.8ml pre-warmed Plate hybridization buffer, and dispense 95 μ l 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 100 μ l 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 200 μ l 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 200 μ l 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 μ l of streptavidin-HRP conjugate in 10ml blocking buffer (1:500) dilution, enough for one plate. Add 95 μ l 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 200 μ l 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 95 μ l 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.

Example of Data Analysis

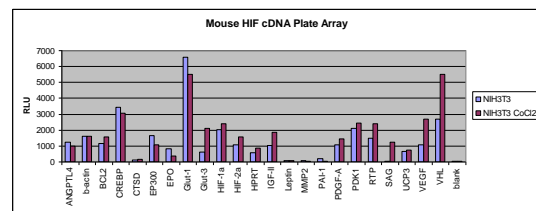


Figure1: Mouse HIF-regulated cDNA plate assay for analysis of 21 genes.

NIH3T3 cells were treated with and without 200 μ M cobalt chloride for 24 hours, RNAs prepared, cDNA synthesized with biotin label and subjected to cDNA plate array hybridization and detection.

Diagram of Mouse HIF-regulated cDNA plate array

	1	2	3	4	5	6	7	8	9	10	11	12
A	ANGPTL4	Glut-3	PDGF-A	ANGPTL4	Glut-3	PDGF-A	ANGPTL4	Glut-3	PDGF-A	ANGPTL4	Glut-3	PDGF-A
B	18SrRNA	HIF-1a	PDK1	18SrRNA	HIF-1a	PDK1	18SrRNA	HIF-1a	PDK1	18SrRNA	HIF-1a	PDK1
C	BCL2	HIF-2a	RTP	BCL2	HIF-2a	RTP	BCL2	HIF-2a	RTP	BCL2	HIF-2a	RTP
D	CREBP	HPRT	SAG	CREBP	HPRT	SAG	CREBP	HPRT	SAG	CREBP	HPRT	SAG
E	CTSD	IGF-II	UCP3	CTSD	IGF-II	UCP3	CTSD	IGF-II	UCP3	CTSD	IGF-II	UCP3
F	EP300	Leptin	VEGF	EP300	Leptin	VEGF	EP300	Leptin	VEGF	EP300	Leptin	VEGF
G	EPO	MMP2	VHL	EPO	MMP2	VHL	EPO	MMP2	VHL	EPO	MMP2	VHL
H	Glut-1	PAI-1	Blank	Glut-1	PAI-1	Blank	Glut-1	PAI-1	Blank	Glut-1	PAI-1	Blank