Signosis
Innovative Plate Assay Solutions

Human HIF-Regulated cDNA Profiling in Cell Lysates

Catalog Number AP-3102 **(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-1alpha 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. Through coupling its CL™ cDNA synthesis kit which allows reverse transcription directly in cell lysates without RNA preparation, Signosis has developed a plate-based hybridization array for profiling the expression of 20+ HIF-regulated genes directly in cell lysates.

Principle of the assay

Unlike conventional reverse transcription which needs total RNA, Signosis has developed a CL™ cDNA synthesis-based plate array. It can synthesize cDNA probes through reverse transcription directly in cell lysates without RNA preparation. The synthesized cDNA probes are then applied for plate array hybridization. Targeted genes are specifically captured onto individual wells on a plate 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.

Material required but not provided

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

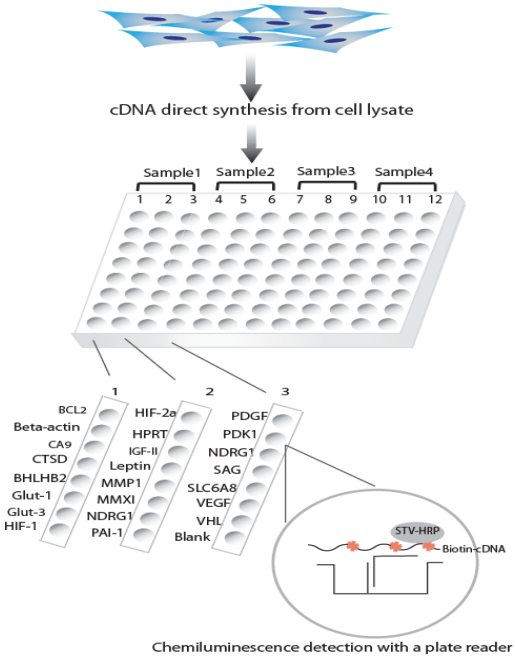

Diagram of human HIF cDNA profiling in cell lysates

Materials provided with the kit

- A 96-well plate coated with 23 different capture oligos (RT)
- Human HIF Reg. Primer Mix (-20 °C)
- Cell lysis Buffer (-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)

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 ^oC 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 4(4).

Assay procedure

1. Sample preparation

(1) Estimate the number of cells. The number of cells should be between 10^4-10^5 cells. Wash the cells with 200ul ice cold 1XPBS and add 100ul ice-cold Cell lysis buffer and then subject to snap-frozen at-80^{o}C. If the cell number is between 2000-10,000 cells, add 20 ul Cell lysis buffer instead.
Notes: Keep the cells on ice during the procedure.
(2) Heat for 75 ^oC for 15 minutes, and put on ice. The cell lysates are ready for use or can be stored at -80 ^oC for the future usage.

2. cDNA synthesis using PCR machine
Note: Briefly spin tubes before opening
(1) Sample preparation
4 ul cell lysate
2 µl Human HIF Reg. Primer Mix
X µl ddH2O

11µl
(2) Incubate for 5 minutes at 65 ^oC, and chill on ice.
(3) Add 8 µl Reverse transcription buffer mix and 1.5µl RT to each reaction tube, and incubate for 1 hour at 45 ^oC.
(4) Heat the reaction to 98 ^oC for 5 minutes, and chill on ice.
(5) The 20ul cDNA is synthesized and labeled with biotin and ready for hybridization on the plate.

3. 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 3ml 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 ^oC 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.***

4. 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:
1 ml Substrate A
1 ml Substrate B
8 ml 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.

Diagram of HIF cDNA plate assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	BCL2	HIF-2a	PDGF-A	BCL2	HIF-2a	PDGF-A	BCL2	HIF-2a	PDGF-A	BCL2	HIF-2a	PDGF-A
B	Beta-actin	HPRT	PDK1	Beta-actin	HPRT	PDK1	Beta-actin	HPRT	PDK1	Beta-actin	HPRT	PDK1
C	CA9	IGF-II	NDRG1	CA9	IGF-II	NDRG1	CA9	IGF-II	NDRG1	CA9	IGF-II	NDRG1
D	CTSD	Leptin	SAG	CTSD	Leptin	SAG	CTSD	Leptin	SAG	CTSD	Leptin	SAG
E	BHLHB2	MMP1	SLC6A8	BHLHB2	MMP1	SLC6A8	BHLHB2	MMP1	SLC6A8	BHLHB2	MMP1	SLC6A8
F	Glut-1	MXI-1	VEGF	Glut-1	MXI-1	VEGF	Glut-1	MXI-1	VEGF	Glut-1	MXI-1	VEGF
G	Glut-3	NDRG2	VHL	Glut-3	NDRG2	VHL	Glut-3	NDRG2	VHL	Glut-3	NDRG2	VHL
H	HIF-1a	PAI-1	Blank	HIF-1a	PAI-1	Blank	HIF-1a	PAI-1	Blank	HIF-1a	PAI-1	Blank