



Human Stem Cell Marker cDNA Plate Array

Catalog Number AP-0181

(For Research Use Only)

Introduction

Stem cells have important characteristics distinguishing from other cell types. They are unspecialized cells capable of renewing themselves through cell division and differentiating into a variety of cell lineages. In addition, they serve as an internal repair system, replenishing specialized cells, in order to maintain the normal turnover of regenerative organs. Identification of a set of stem cell –specific genes can function as stem cell molecular marker to facilitate stem cell research. Signosis' Stem cell cDNA Plate Array allows profiling 30+ 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.

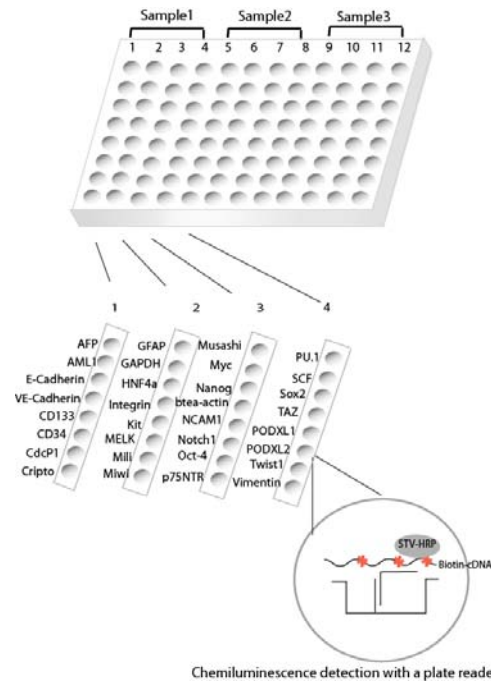


Diagram of human stem cell marker cDNA plate array

Materials provided with the kit

- A 96-well plate coated with 31+ different capture oligos (RT)
- Human Stem Cell 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)

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 μ l 1-10 μ g total RNA
 2 μ l Human Stem Cell 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 3 repeat sections, Column 1-4, 5-8, 9-12 for 3 different samples.
- (3) Mix 20 μ l cDNA with 3.5ml 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.
- (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 300 μ l 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 200 μ l 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 μ l 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 200 μ l 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 95 μ l substrate solution to each well and incubate for 1 minutes.
- (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 human stem cell marker

cDNA plate array

	1	2	3	4	5	6	7	8	9	10	11	12
A	AFP	GFAP	Musashi	PU.1	AFP	GFAP	Musashi	PU.1	AFP	GFAP	Musashi	PU.1
B	AML1	GAPDH	Myc	SCF	AML1	GAPDH	Myc	SCF	AML1	GAPDH	Myc	SCF
C	E-Cadherin	HNF4a	Nanog	Sox2	E-Cadherin	HNF4a	Nanog	Sox2	E-Cadherin	HNF4a	Nanog	Sox2
D	VE-Cadherin	Integrin b1	Beta-actin	TAZ	VE-Cadherin	Integrin b1	Beta-actin	TAZ	VE-Cadherin	Integrin b1	Beta-actin	TAZ
E	CD133	Kit	NCAM1	PODXL1	CD133	Kit	NCAM1	PODXL1	CD133	Kit	NCAM1	PODXL1
F	CD34	MELK	Notch1	PODXL2	CD34	MELK	Notch1	PODXL2	CD34	MELK	Notch1	PODXL2
G	CdcP1	Mili	Oct-4	Twist1	CdcP1	Mili	Oct-4	Twist1	CdcP1	Mili	Oct-4	Twist1
H	Cripto	Miwi	p75NTR	Vimentin	Cripto	Miwi	p75NTR	Vimentin	Cripto	Miwi	p75NTR	Vimentin