



Single Stem Cell Gene Expression Assay Kit

Catalog Number SC-0001

(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. A set of stem cell-associated genes can function as stem cell biomarkers, which facilitate stem cell research. However, limit of stem cell resources hampers its accessible measurement of these genes. Signosis has developed single stem cell gene expression assay kit by combining PCR and signal amplification together, which dramatically increase the sensitivity of detection. The kit can be used for analyzing 8 stem cell-associated gene expression simultaneously.

Principle of the assay

Signosis has developed a high sensitive detection method for profiling single-cell gene expression through two level amplifications: template amplification and signal amplification. The RNAs in cell lysate is first reverse transcribed into cDNA, followed by PCR amplification. The amplified DNA then is captured on a 96-well plate. During detection, the signal is further amplified with a multiple-biotin molecule and then 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)

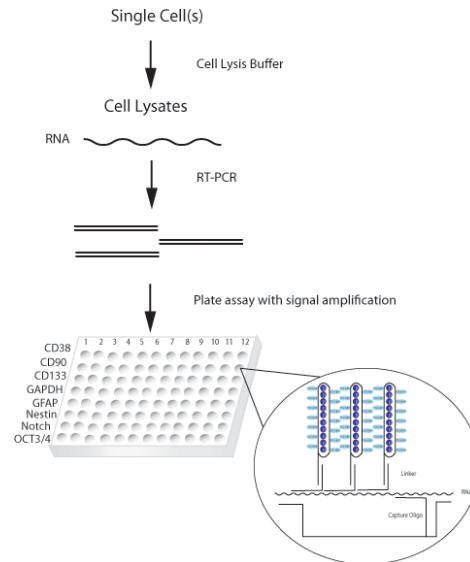


Diagram of single stem cell gene expression assay kit.

Materials provided with the kit

- A 96-well plate coated with capture oligo (RT)
- 8 individual PCR primer pairs (-20 °C)
- 8 Capture oligos (-20 °C)
- Cell lysis Buffer (-20 °C)
- RNA amplification buffer mix (-20 °C)
- Reverse transcriptase RT (-20 °C)
- PCR buffer mix (-20 °C)
- Multiple-biotin molecules (-20 °C)
- Streptavidin-HRP conjugate (4 °C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (RT)
- 5x Detection 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 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.
- Dilute 1000 times of streptavidin-HRP with blocking buffer before use at Step 4(4).

Assay procedure

1. Sample preparation

- (1) Estimate the number of cells. Wash the cells with 200ul ice cold 1XPBS. If the range of cell number is from 1000 to 10,000, and add 50ul ice-cold Cell lysis buffer and then subject to snap-frozen at -80°C. If the range of cell number is from 100-1000, add 20 ul Cell lysis buffer. The cell number is 1- 100, add 5 ul Cell lysis buffer.

Notes: Keep the cells on ice during the procedure.

- (2) Heat for 75 °C for 15 minutes, and put on ice. The cell lysates are ready for use or can be stored at -80 °C for the future usage.

2. cDNA synthesis using PCR machine

Note: Briefly spin tubes before opening

- (1) Sample preparation
 - 2 ul cell lysate
 - 2 ul RT primer
 - 2 ul Reverse transcription buffer mix
 - 1ul Reverse transcriptase
 - 3ul ddH₂O
- (2) Incubate for 30 minutes at 45 °C
- (3) For each reaction, add 1 µl cDNA, 1 µl the corresponding primer and 0.5 ul DNA polymerase to 15 µl PCR buffer

Notes: Make a master mix of water, buffer, DNA polymerase, and cDNA template (if using the same template for all reactions), aliquot into individual reactions and add primers separately.

- (4) Proceed PCR cycles (32 cycles)
 - 94 °C 30 seconds
 - 55 °C 30 seconds
 - 72 °C 30 seconds

3. Plate hybridization

- (1) Remove the sealing film
- (2) Arrange the appropriate number of the wells of the plate based on your experiment. Each column for one sample.
- (3) Mix 2ul PCR products and 2ul Multiple-biotin molecules with 100ul pre-warmed MBSA Hybridization Buffer on a well.

- (4) Transfer 2ul of capture oligo to the corresponding wells.
- (5) Seal the whole plate with foil film (provided) securely and incubate the plate at 45 °C for 3-hours. 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.

4. Plate detection

- (1) Remove the foil from the top of the assay wells. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200µl of pre-warmed 1x Plate Hybridization Wash Buffer.
- (2) Add 100ul MBSA Hybridization Buffer and 4ul Multiple-biotin molecules, seal the wells with sealing foil securely and incubate the plate at 45°C for 1 hour.
- (3) Remove the foil from the top of the assay wells. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200µl of pre-warmed 1x Plate Hybridization Wash Buffer.
- (4) Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- (5) Add 200µl of Blocking buffer and incubate for 15 minutes at room temperature with gentle shaking.
- (6) Invert the plate over an appropriate container to remove block buffer. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- (7) Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 20 min at room temperature with gentle shaking.
- (8) 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.
- (9) Repeat (8) for additional 2 time washes.
- (10) Freshly prepare the substrate solution
For the whole plate:
 - 1 ml Substrate A
 - 1 ml Substrate B
 - 8 ml Substrate dilution buffer
- (11) Add 95µl substrate solution to each well and incubate for 1minutes.
- (12) 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

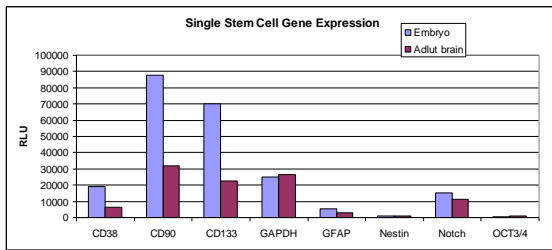


Figure: Analysis of 8 gene expression in mouse embryo and brain with Single Stem Cell Gene Expression Assay Kit. The RNA in 10 cells is first reverse transcribed into cDNA, followed by PCR amplification. The amplified DNA is captured on a 96-well plate and detected with a multiple-biotin molecule and then streptavidin-HRP.

Single Stem Cell Gene Expression

	1	2	3	4	5	6	7	8	9	10	11	12
A	CD38	CD38	CD38	CD38	CD38	CD38	CD38	CD38	CD38	CD38	CD38	CD38
B	CD90	CD90	CD90	CD90	CD90	CD90	CD90	CD90	CD90	CD90	CD90	CD90
C	CD133	CD133	CD133	CD133	CD133	CD133	CD133	CD133	CD133	CD133	CD133	CD133
D	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH	GAPDH
E	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP	GFAP
F	Nestin	Nestin	Nestin	Nestin	Nestin	Nestin	Nestin	Nestin	Nestin	Nestin	Nestin	Nestin
G	Notch	Notch	Notch	Notch	Notch	Notch	Notch	Notch	Notch	Notch	Notch	Notch
H	OCT3/4	OCT3/4	OCT3/4	OCT3/4	OCT3/4	OCT3/4	OCT3/4	OCT3/4	OCT3/4	OCT3/4	OCT3/4	OCT3/4