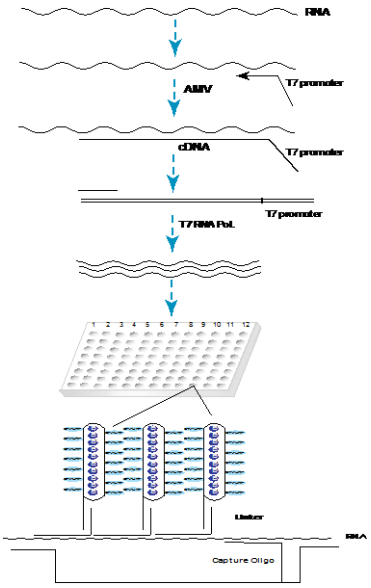
Signosis
Innovative Plate Assay Solutions

Human LncRNA Expression Plate Assay

Catalog Number LA-0001 **(For Research Use Only)**

Introduction

Protein-coding genes represent only 2% of the human genome and a fraction of what can be transcribed. The genome also encodes many short and long noncoding RNA (lncRNA) molecules. LncRNA vary in size from several hundred to tens of thousands of bases and have recently emerged as key regulators in many biological processes. LncRNAs are regulated during development and in response to diverse signaling pathways and can be misexpressed in cancer and other diseases. For instance, Hotair is highly expressed in breast cancer metastases and in primary tumors predisposed to future metastases. Although over 3000 human lncRNAs have been identified, less than 1% have been further characterized, with only a few dozen being well characterized, with known distinct gene expression patterns in primary tumors, metastases and other diseases. Signosis has developed a simple plate assay for profiling of the expression of 15 well-characterized lncRNAs with high sensitivity and specificity.



LncRNA expression plate assay

Principle of the assay

In the assay, the total RNAs are transcribed with T7 promoter-tagged primers. The cDNAs are then subjected to the first template amplification with T7 RNA polymerase. The amplified RNAs are then subjected to secondary amplification - multiple biotin signal amplification (MBSA). In MBSA, a polymer amplifier containing multiple biotins binds to the targeted RNA via hybridization of a linker. The linker contains two moieties, one binds to the polymer and another to the targeted molecule. A set of linkers are designed that shares a common tag sequence, although they contain different target sequences to hybridize different regions of the targeted molecule. This design leads to the binding of multiple biotin polymers to multiple regions. The captured biotin molecule is last detected with streptavidin-HRP and a chemiluminescent substrate.

Materials provided with the kit

- One 96-well plate (RT)
- 1.2X amplification buffer
- 16 linker detection oligos in 2X8well strip.
- Multiple-biotin molecules (-20^{o}C)
- Plate Hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (RT)
- 5x Detection wash buffer (RT)
- Streptavidin-HRP conjugate (4^{o}C)
- Substrate A (4^{o}C)
- Substrate B (4^{o}C)
- Substrate dilution buffer (RT)
- Sealing foils

Instrument and materials required

- Hybridization incubator
- Shaker
- Plate reader for chemiluminescent detection

Reagent preparation before starting experiment

- Warm up Plate hybridization buffer and 5x Plate Hybridization Wash buffer at 42°C before use.
- Dilute 30 ml of 5x Plate Hybridization wash buffer with 120 ml of dH_2O before use.
- Dilute 40 ml of 5x Detection wash buffer with 160 ml of dH_2O before use.
- Dilute streptavidin-HRP 1:1000 in blocking buffer before use at Step 11.

Assay procedure

1. Determine the appropriate number of the wells to use for your experiment. There are 6 sections in one plate and each section is for one sample. Remove the top foil sealing film with a blade to expose the experimental sections. Make sure the rest of wells remain well sealed.

2. Prepare the first amplification reaction.
   Mix the following component together:
   1µl of RNA (200 ng-2 µg)
   18 µl 1.2 Amplification buffer
   1µl enzyme mix
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   20 µl Total Volume
   Incubate the reaction at 42°C for 90 minutes.

3. Incubate the amplified product on the plate.
   3.1 Mix the 20 µl reaction in 1.7 ml Plate hybridization buffer.
   3.2 Add 100 µl to each well within one section.
   3.3 Using a 8-channel pipettor, transfer 2 µl detection oligo from each well of the strips to the corresponding columns in the plate.

4. Cut new sealing foil (provided) to fit the area of the experimental wells and seal the wells securely. 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. Incubate the plate at 42 °C for 2 hours to overnight.

5. Remove the foil from the top of the assay wells. Invert the plate over an appropriate container and expel the contents forcibly, then firmly tap against clean paper towels. Wash the plate 3 times by adding 200 µl of pre-warmed 1x Plate Hybridization Wash Buffer.

6. Add 100 µl Plate Hybridization Buffer and 4 µl Multiple-biotin molecules, cut new sealing foil and seal the wells securely. Incubate the plate at 42 °C for 1 hour.

7. Repeat the washing step as described in step 5.

8. Add 200 µl of Blocking buffer and incubate for 15 minutes at room temperature with gentle shaking.

9. Invert the plate over an appropriate container and expel the contents forcibly, then firmly tap against clean paper towels.

10. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.

11. Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times with 200 µl 1X Detection wash buffer for 5 min at room temperature with gently shaking. Completely remove liquid at each wash by firmly tapping the plate against clean paper towels.

12. Freshly prepare the substrate solution.
    For the whole plate:
    1 ml Substrate A
    1 ml Substrate B
    8 ml Substrate dilution buffer

13. Add 95 µl substrate solution to each well and incubate for 1 minute.

14. 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.

	1	2	3	4	5	6	7	8	9	10	11	12
A	HULC	UCA1	HULC	UCA1	HULC	UCA1	HULC	UCA1	HULC	UCA1	HULC	UCA1
B	XIST	PCA3	XIST	PCA3	XIST	PCA3	XIST	PCA3	XIST	PCA3	XIST	PCA3
C	CDKN	ANRIL	CDKN	ANRIL	CDKN	ANRIL	CDKN	ANRIL	CDKN	ANRIL	CDKN	ANRIL
D	NEAT1	GAPDH	NEAT1	GAPDH	NEAT1	GAPDH	NEAT1	GAPDH	NEAT1	GAPDH	NEAT1	GAPDH
E	DLEU1	PCGEM1	DLEU1	PCGEM1	DLEU1	PCGEM1	DLEU1	PCGEM1	DLEU1	PCGEM1	DLEU1	PCGEM1
F	MEG3	GAS5	MEG3	GAS5	MEG3	GAS5	MEG3	GAS5	MEG3	GAS5	MEG3	GAS5
G	NRON	Hotair	NRON	Hotair	NRON	Hotair	NRON	Hotair	NRON	Hotair	NRON	Hotair
H	H19	Malat1	H19	Malat1	H19	Malat1	H19	Malat1	H19	Malat1	H19	Malat1