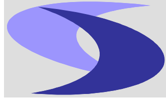

**Signosis**
BioSignal Capture

# Rat PPARr-Regulated cDNA Plate Array

**Catalog Number AP-1161** **(For Research Use Only)**

## Introduction

The peroxisome-proliferator activated receptor gamma (PPARr) is a member of the nuclear receptor superfamily of transcription factors. PPARr is important to a variety of biological processes, such as adipocyte differentiation, glucose homeostasis, lipid trafficking as well as vascular function and hypertension. PPARr exerts its effects by regulating target gene transcription in a ligand-dependent manner. Upon activation, the PPARγ-RXR heterodimer binds to specific DNA sequences on the target gene promoter and leads to transcriptional regulation. Signosis developed a plate-based array for profiling 20+ PPARr-regulated genes.

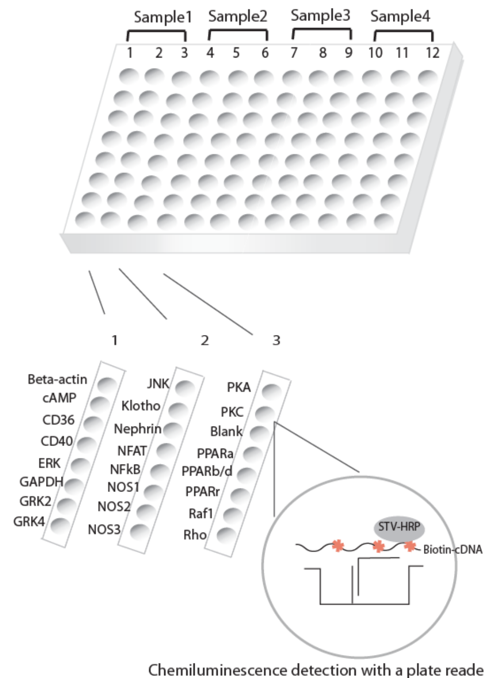


Diagram of rat PPARr-regulated cDNA plate array

## 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 31+ different capture oligos (RT)
- Rat RRARr Reg. Primer Mix (-20 $^{o}$C)
- Reverse transcription buffer mix (-20 $^{o}$C)
- Reverse transcriptase RT (-20 $^{o}$C)
- Streptavidin-HRP conjugate (4$^{o}$C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Block buffer (RT)
- 5xDetection wash buffer (RT)
- Substrate A (4$^{o}$C)
- Substrate B (4$^{o}$C)
- Substrate dilution buffer (RT)

## Material required but not provided

PCR machine
Incubator
0.2ml PCR tube
Plate reader for chemiluminescence detection
ddH2O (RNAase free)

## Reagent preparation before starting experiment

- Dilute 30ml of 5x Plate hybridization wash buffer with 120 ml of d$H_2O$ before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of d$H_2O$ before use.
- Warm up Plate hybridization buffer and Hybridization Wash buffer at 45 $^{o}$C before use.
- Dilute 1000 times of streptavidin-HRP with block buffer before use at Step 3(3).

## Assay procedure

**1. cDNA synthesis**

**Note: Briefly spin tubes before opening**

(1) Sample preparation

X µl 1-10µg total RNA

2 µl Rat PPARr Reg. Primer Mix

X µl ddH2O

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11µl

(2) Incubate for 5 minutes at 65 $^{o}$C, and chill on ice.

(3) Add 8 µl Reverse transcription buffer mix and 1µl RT to each reaction tube, and incubate for 1 hour at 45 $^{o}$C.

(4) Heat the reaction to 98$^{o}$C for 5 minutes, and chill on ice.

(5)The 20ul cDNA is synthesized and labeled with biotin, ready for hybridization on the plate.

**2. Plate hybridization**

(1) Arrange the appropriate number of the wells of the plate based on your experiment by removing the top foil sealing film with a blade. Keep the unused well sealed. The whole plate is divided into 4 repeat sections, Column 1-3, 4-6, 7-9, 10-12 for 4 different samples.

**Note: keep the removed sealing film for resealing during hybridization**

(2) Mix 20ul cDNA with 2.8ml 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.

(3) Seal the wells with foil film securely and incubate the plate at 45 $^{o}$C for overnight. Ensure the numbers and letters on the plate are clearly visible from under foil seal.

**3. Plate detection**

(1) 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.

Optional: To ensure remove all traces of the buffer in last washing step, invert the plate on a clean, dry paper towel and centrifuge at 240xg for 1 minute at room temperature

(2) Add 200µl of Block buffer incubate for 15 minutes at room temperature with gentle shaking.

(3) Invert the plate over an appropriate container to remove block buffer. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.

(4) 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.

(5) Repeat Step (4) for additional 2 time washes.

(6) Freshly prepare the substrate solution

For the whole plate:

1ml Substrate A

1ml Substrate B

8ml Substrate dilution buffer

(7) Add 95µl substrate solution to each well and incubate for 1minutes.

(8) Determine the chemiluminescence of each well

## Diagram of rat PPAR-regulated cDNA plate array

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| B-actin | JNK | PKA | B-actin | JNK | PKA | B-actin | JNK | PKA | B-actin | JNK | PKA |
| cAMP | Klotho | PKC | cAMP | Klotho | PKC | cAMP | Klotho | PKC | cAMP | Klotho | PKC |
| CD36 | Nephrin | Blank | CD36 | Nephrin | Blank | CD36 | Nephrin | Blank | CD36 | Nephrin | Blank |
| CD40 | NFAT | PPARa | CD40 | NFAT | PPARa | CD40 | NFAT | PPARa | CD40 | NFAT | PPARa |
| ERK | NFKB | PPARr | ERK | NFKB | PPARr | ERK | NFKB | PPARr | ERK | NFKB | PPARr |
| GAPDH | NOS1 | PPARß/d | GAPDH | NOS1 | PPARß/d | GAPDH | NOS1 | PPARß/d | GAPDH | NOS1 | PPARß/d |
| GRK-2 | NOS2 | Raf-1 | GRK-2 | NOS2 | Raf-1 | GRK-2 | NOS2 | Raf-1 | GRK-2 | NOS2 | Raf-1 |
| GRK-4 | NOS3 | Rho | GRK-4 | NOS3 | Rho | GRK-4 | NOS3 | Rho | GRK-4 | NOS3 | Rho |