Signosis
BioSignal Capture

ER Filter Plate Assay

Catalog Number FA-0003 **(For Research Use Only)**

Introduction

ERs (ERalpha and ERbeta) mediate cellular signaling of estrogens, belonging to the nuclear receptor family of transcription factors. The mechanism of regulatory actions of ERs is that ligand-bound ERs bind directly to estrogen response elements (ERE), in the promoters of target genes or via protein-protein interactions with other transcription factors. The dysfunction of ERs has been demonstrated to be closely related with breast cancer, prostate cancer and lung cancer. Signosis has developed ER filter assay for monitoring DNA binding activity of ER. Unlike conventional gel shift assays, ER filter assay is a high throughput assay and it can analyze multiple samples simultaneously. Meanwhile, the assay can be set up for a few samples with less tedious than non-radioactive gel shift assay or much safe than ^{32}P-based gel shift assay.

Principle of the assay

Signosis' ER filter assay is a plate-based analysis for monitoring the activity of ER. In the assay, biotin labeled DNA binding sequence of ER is mixed with nuclear extract to allow formation of ER-DNA complex. A filter plate is used to retain bound ER probe and remove free DNA probe. The bound pre-labeled ER probe is then eluted from the filter and hybridize to the corresponding well of 96 well of Hybridization Plate for quantitative analysis. The captured ER probe is further detected with streptavidin-HRP. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

Materials provided with the kit

- One 96-well Hybridization Plate (RT)
- One 96-well Filter Plate (RT)
- TF (transcription factor) binding buffer mix (-20 °C)
- ERprobes (-20 °C)
- Filter binding buffer (4 °C)
- Filter wash buffer (4 °C)
- Elution buffer (RT)
- 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)

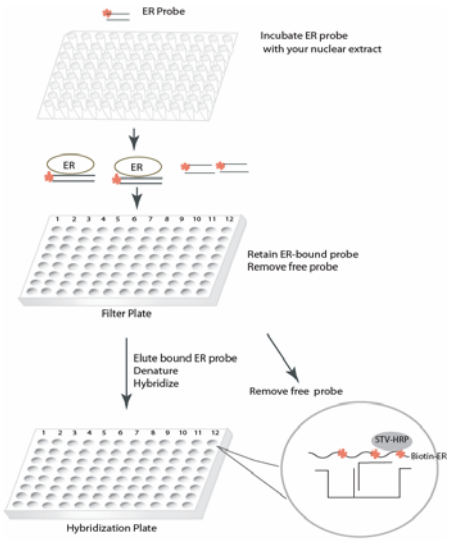

Diagram of ER Filter Plate Assay

Material required but not provided

- Nuclear Extraction Kit from Signosis (SK-0001)
- Wash Collection Plate (Any 96-well ELISA plate or equivalent 96-well plastic plate)
- 96-well PCR plate
- PCR machine
- Microplate centrifuge working at 4 °C
- Hybridization incubator
- Shaker
- Plate reader for luminescent detection
- ddH2O (RNAase free)

Reagent preparation before starting experiment

- Warm up Plate Hybridization Buffer and Hybridization Wash buffer at 45 °C before use.
- 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.
- Dilute 500 times of streptavidin-HRP with blocking buffer before use.

Assay Procedure

TF DNA Complex Formation

1. Mix the following components for each reaction in a tube or one well of a PCR plate
   10ul TF Binding Buffer Mix
   2ul ERE probe
   Xul nuclear extract (2µg-10µg)
   Xul ddH2O
   20ul
2. Incubation at 16°C for 30 minutes in a PCR machine

Separation of TF DNA Complex from Free Probes

1. Based on the number of experiment, allocate the number of wells on Filter Plate, and remove the top foil sealing film with a blade. Keep the unused well sealed.
2. Place Wash Collection Plate on the bottom of Filter Plate during the following steps to assemble Filter Plate/Wash Collection Plate.
3. Pre-wet the Filter Plate
   i. Add 250ul/well cold Filter binding buffer to each experimental well of Filter Plate.
   ii. Centrifuge Filter Plate/Wash Collection Plate at 600g for 1 minute, and discard the flow-through from Wash Collection Plate.
4. Add 20ul cold Filter binding buffer to each TF-DNA complex reaction tube or well, and transfer 35ul of the mixture to the center of the filter in the corresponding well of Filer Plate.
5. Incubate on ice for 30 minutes.
   Don't incubate longer than 30 minutes, which results in high background.
6. Centrifuge Filter Plate/Wash Collection Plate at 600g for 2 minutes and discard the flow-through from the Wash Collection Plate.
7. Add 250ul cold Filter wash buffer to each experimental well of Filter Plate and incubate for 2-3 minutes on ice.
8. Centrifuge Filter Plate/Wash Collection Plate at 600g for 2 minutes, and discard the flow-through from Wash Collection Plate.
9. Repeat the step 7-8 for 3 times for total 4 washes.

Elution of Bound Probe

1 Add 60ul of Elution buffer to the center of each experimental well in Filter Plate
2 Place a 96-well PCR plate on the top of Wash Collection Plate or a 96-well PCR plate rack.
3 Place Filter Plate on the top of a 96-well PCR plate. In order to collect the elution completely and facilitate the centrifugate, ensure each well on the top plate matches the bottom ones, and fix the assemblies with tape on sides of the plates.
4 Incubate for 5 minutes at room temperature.
5 Centrifuge assemblies at 600g for 2 minutes. The 60 ul of sample should be in each well of the 96 well PCR plate.
6 Denature the samples in the Elution Plate at 95 °C for 3 minutes in a PCR machine, and transfer on ice immediately. The samples are ready for hybridization or store -20 °C for the future use.

Hybridization of Eluted Probe with Hybridization Plate

1. Based on the number of experiment, allocate the number of the wells of Hybridization Plate and remove the top sealing film.
2. Add 60ul Hybridization buffer to each experimental well of TF Hybridization Plate, transfer 30ul of denatured sample to the corresponding well, and mix gently.
3. Seal the wells with foil film securely and hybridize at 42 °C for 3 hours to 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.*

Detection of Bound Probe

1. Remove the foil film fro the experimental wells with a blade. Keep the unused well sealed.
2. Invert the Hybridization 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.
3. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
4. Add 200µl of Blocking buffer incubate for 15 minutes at room temperature with gentle shaking.
5. Invert the plate over an appropriate container to remove blocking buffer.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. 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.
8. Repeat Step 7 for additional 2 time washes.
9. Freshly prepare the substrate solution:
   For the whole plate:
   1ml Substrate A
   1ml Substrate B
   8ml Substrate dilution buffer
10. Add 95µl substrate solution to each well and incubate for 1 min.
11. 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.

Data Example

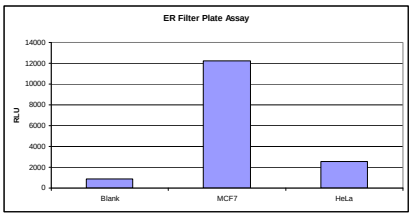

Figure. ER filter assay analysis of DNA binding activity of ER in MCF7 and HeLa. Nuclear extract were prepared, and 2ug of nuclear extract were used for ER filter assay. The ERE-bound probe was hybridized with the ER hybridization plate. The chemiluminescent signal was measured with a plate reader.