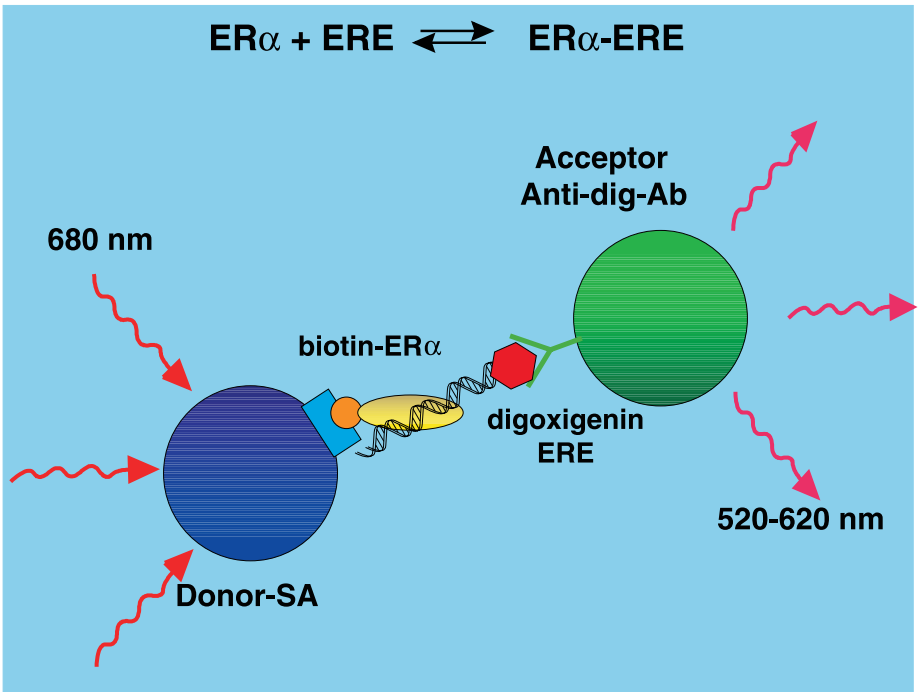
Technical Note

AlphaScreen™ AN004-ASc

ERα Binding Assay



The AlphaScreen™ ERα binding assay has been designed to directly measure the inhibition of ERα binding to estrogen responsive elements (ERE) following exposure to various chemicals. The assay is based on the capture of biotin-ERα and digoxigenin-ERE by streptavidin-donor and anti-digoxin acceptor beads respectively. The AlphaScreen ERα binding assay is specific and reliable. This assay is highly competitive with existing ERα binding assay in terms of ease of use, dynamic range, signal-to-noise ratio (SNR) and time to completion.

The AlphaScreen ERα binding assay is a highly sensitive, homogeneous and non radioactive screening assay. The assay is miniaturized, fully automatable and can be performed in 1 hour.

Technology from:

BioSignal Packard
A Packard BioScience Company

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Example #1:

Competition of digoxigenin-ERE-1 binding to biotin-ERα
developed in Packard 384-well OptiPlate™ microplates
(product # 6005214)

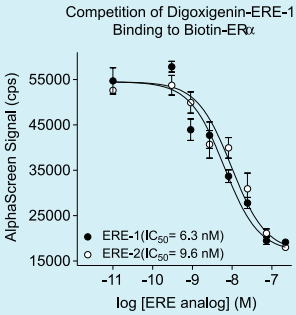
Reagents

1. Anti-digoxin-acceptor beads (5 mg/ml in 25 mM HEPES pH 7.4) dilute to 100 µg/mL in assay buffer
2. Streptavidin-donor beads (5 mg/mL in 25 mM HEPES pH 7.4) dilute to 100 µg/mL in assay buffer
3. Digoxigenin-ERE-1 (50 µM in PBS) dilute to 7.5 nM in assay buffer with anti-digoxin-acceptor dilution
4. Biotin-ERα (10 µM in 25 mM HEPES pH 7.4, 0.1% CHAPS) dilute to 10 nM with anti-digoxin-acceptor beads/ digoxigenin-ERE-1 mix
5. ERE-1 or ERE-2 analogs (100 µM in PBS) dilute to 1 nM – 100 µM in assay buffer

Assay buffer: PBS + 0.1% BSA

Protocol:

1. Add: 5 µL ERE-1 or ERE-2
   15 µL digoxigenin-ERE-1/ biotin-ERα-anti-digoxigenin acceptor beads mix
   – incubate 45 minutes at RT
2. Add 5 µL donor beads
   – incubate 15 minutes at RT
3. Read plate



Example #2:

Saturation of ERE-1 by ERα
developed in Packard 384-well OptiPlate™ microplates
(product # 6005214)

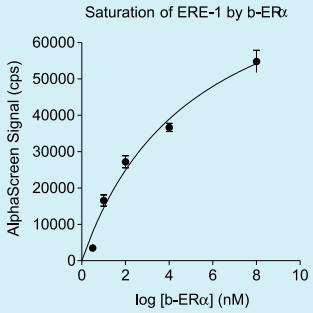
Reagents:

1. Anti-digoxin-acceptor beads (5 mg/ml in 25 mM HEPES pH 7.4) dilute to 50 µg/mL in assay buffer
2. Streptavidin-donor beads (5 mg/mL in 25 mM HEPES pH 7.4) dilute to 100 µg/mL in assay buffer
3. Digoxigenin-ERE-1 (50 µM in PBS) dilute to 7.5 nM in assay buffer with anti-digoxigenin acceptor beads dilution
4. Biotin-ERα (10 µM in 25 mM HEPES pH 7.4, 0.1% CHAPS) dilute to 2.5 - 40 nM in assay buffer

Assay buffer: PBS + 0.1% BSA

Protocol:

1. Add: 5 µL biotin-ERα, 15 µL digoxygenin-ERE-1/acceptor beads
   – incubate 45 minutes at RT
2. Add 5 µL donor beads
   – incubate 15 minutes at RT
3. Read plate



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