FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

Intended Use

This immunoassay kit allows for the specific measurement of human Estrogen Receptor, ER concentrations in cell culture supernates, serum, and plasma.

Introduction

The estrogen receptor (ER) is a member of the nuclear hormone family of intracellular receptors which is activated by the hormone 17 β-estradiol. The main function of the estrogen receptor is as a DNA binding transcription factor which regulates gene expression. However the estrogen receptor also has additional functions independent of DNA binding.

There are two different forms of the estrogen receptor usually referred to as α and β each encoded by a separate gene (ESR1 and ESR2 respectively). Hormone activated estrogen receptors form dimers, and since the two forms are coexpressed in many cell types, the receptors may form ERα (αα) or ERβ (ββ) homodimers or ERαβ (αβ) heterodimers. Estrogen receptor alpha and beta show significant overall sequence homology, and both are composed of seven domains. The ERα is found in endometrium, breast cancer cells, ovarian stroma cells and in the hypothalamus. The expression of the ERβ protein has been documented in kidney, brain, bone, heart, lungs, intestinal mucosa, prostate, and endothelial cells. While they both bind estrogen as well as other agonists and antagonists, the two receptors have distinctly different localizations and concentrations within our body. Structural differences also exist between the two. The ER ligands tamoxifen, raloxifene, and ICI-164384 were activators with Erβ as well as ERα, although the degree of agonism differed between cell types. These molecules are examples of SERM's, selective estrogen receptor modulators. Thus, the role of estrogen complexed to ERβ appears to be to turn off transcription of these genes, whereas the SERMs may override this blockade and activate gene transcription.

Estrogen receptor structure-function is a vast topic and the subject of very active current research. Nuclear receptors are a large family of structurally related ligand-inducible transcription factors, including steroid receptors (SRs), thyroid/retinoids receptors (TR, RARs and RXRs), vitamin D receptors (VDR), LXR, PPARs, estrogen receptors (ERα and ERβ), and orphan receptors for which no ligand has been yet identified. While having in common a modular structure, they are activated by distinct lipophilic small molecules such as glucocorticoids, progesterone, estrogens, retinoids, and fatty acid derivatives.All nuclear receptors have a hydrophobic pocket into which its specific ligand binds, with helix 12 (H12) being the key response element of NR's. When an agonist is bound to a NR, H12 is oriented anti-parallel to H11, capping the ligand binding pocket.

Test Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Estrogen Receptor has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Estrogen Receptor present is bound by the immobilized antibody. An enzyme-linked polyclonal antibody specific for Estrogen Receptor is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Estrogen Receptor bound in the initial step. The color development is stopped and the intensity of the color is measured.

Materials and Components

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	1 X 20ml
Assay Diluent A	1 X 10ml
Assay Diluent B	1 X 10ml
Detection Reagent A	1 X 120µl
Detection Reagent B	1 X 120µl
Wash Buffer (25 x concentrate)	1 X 30ml
Substrate	1 X 10ml
Stop Solution	1 X 10ml

Sample Collection and Storage

Cell culture supernates

Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Serum

Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000×g. Remove serum and assay immediately or aliquot and store samples at -20℃.

Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8℃ within 30 minutes of collection. Store samples at ≦ -20℃. Avoid repeated freeze-thaw cycles.

Note: Citrate plasma has not been validated for use in this assay.

Limitations of the Procedure

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

Reagent Preparation

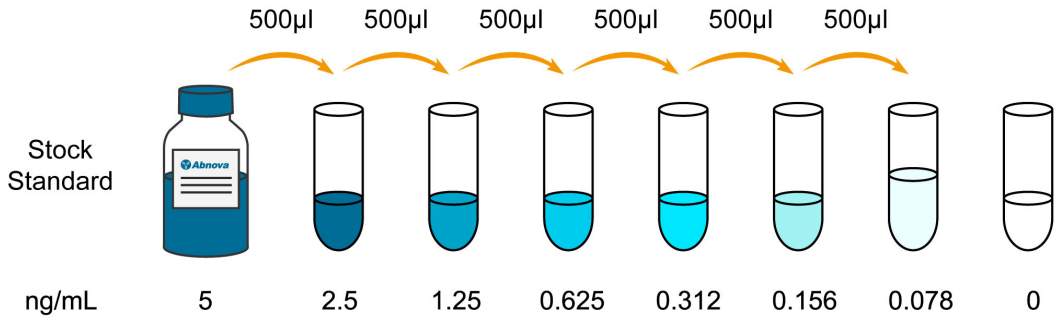
Bring all reagents to room temperature before use.

Wash Buffer

If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Standard

Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 5 ng/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the highest standard (5 ng/ml). The Sample Diluent serves as the zero standard (0 ng/ml).



Detection Reagent A and B

Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively.

Assay Procedure

Allow all reagents to reach room temperature. Arrange and label required number of strips.

1. Prepare all reagents, working standards and samples as directed in the previous sections.
2. Add 100 µL of Standard, Control, or sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37℃.
3. Remove the liquid of each well, don't wash.
4. Add 100 µL of Detection Reagent A to each well. Incubate for 1 hour at 37℃. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
5. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Reagent B to each well. Cover with a new adhesive strip. Incubate for 1 hours at 37℃.
7. Repeat the aspiration/wash as in step 5.
8. Add 90 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light.
9. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

Specificity

This assay recognizes recombinant and natural human Estrogen Receptor. No significant cross-reactivity or interference was observed.

Important Note

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.
4. When mixing or reconstituting protein solutions, always avoid foaming.

5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Estrogen Receptor concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Storage of Test Kits and Instrumentation

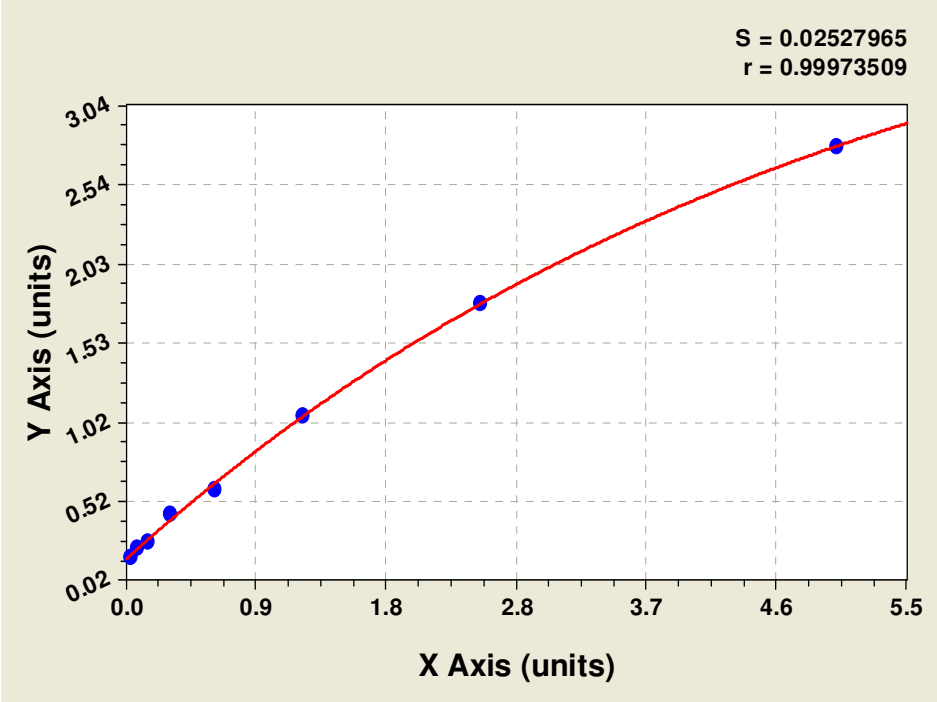
1. Unopened test kits should be stored at 2-8℃ upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Certificate of Analysis

Results of a typical standard curve are provide for demonstration only and should not be used to obtain test results. A standard curve must be run for each set of samples assayed.



X: Standard Concentration (ng/ml), Y: O.D. Value 450nm

Performance Characteristics

Intra-Assay CV: <3.9%

Inter-Assay CV: <7.7%

Spike Recovery: 97.8%

Quick Reference Procedure

IMPORTANT: Please refer to the supplied protocol for preparation of Working Stocks as noted below.

Human Estrogen Receptor, ER Standard Prepaparation (step 2)

Tube	Working Stock	S_7	S_6	S_5	S_4	S_3	S_2	S_1	BL
Amount Taken from Tube to Left(ml)	---	0.020	0.5	0.5	0.5	0.5	0.5	0.5	---
Sample Diluent	---	0.98	0.5	0.5	0.5	0.5	0.5	0.5	1.0
Final Conc.(ng/ml)	5	5	2.5	1.25	0.625	0.312	0.156	0.078	0

Antibody Concentrate Preparation (Step 5)

Microplate Strips Used	2	4	6	8	10	12
Working Stock(µl)	20	40	60	80	100	120
Conjugate Diluent(ml)	2.0	4.0	6.0	8.0	10.0	12.0

HRP Conjugate Concentrate (step 7)

Microplate Strips Used	2	4	6	8	10	12
Working Stock(µl)	20	40	60	80	100	120
Conjugate Diluent(ml)	2.0	4.0	6.0	8.0	10.0	12.0