ANTI-ESTROGEN RECEPTOR-β (ER β)
Developed in Rabbit, IgG fraction of Antiserum

Product Number E 0901

Product Description
Anti-Estrogen-Receptor-β (ER β) is developed in rabbit using a synthetic peptide (YAEPKSPWCEARSLEHT) as immunogen. The sequence represents amino acids 54-71 of rat and mouse estrogen receptor β (ER β) and amino acid 46-63 of human estrogen receptor β. The IgG fraction of antiserum is purified using protein A.

Anti-Estrogen-Receptor-β (ER β) specifically recognizes estrogen receptor β (65 kDa) by immunoblotting. It does not detect the estrogen receptor α. The antibody recognizes the ER β in rat and human and due to sequence homology is expected to recognize mouse ER β, but this has not been tested.

Anti-Estrogen-Receptor-Beta may be used to detect and localize the ER β by immunoblotting, Immunoprecipitation, and Immunohistochemistry (formalin-fixed, paraffin-embedded section). It has been reported to work on frozen rat ovary sections.

The discovery of a second estrogen receptor has redefined the estrogen signaling pathway and may have broad implications on estrogen-responsive tissues.1 The new estrogen receptor, named estrogen receptor-beta (ERbeta), is preferentially expressed in the prostate and maintains some characteristics that are different from ERalpha.2 The rat tissue distribution and/or the relative level of ERalpha and ERbeta expression seems to be quite different, i.e., moderate to high expression in uterus, testis, pituitary, ovary, kidney, epididymis, and adrenal for ERalpha and prostate, ovary, lung, bladder, brain, bone, uterus, and testis for ERbeta. Within the same organ it often appears that the ER subtypes are expressed in different cell types, supporting the hypothesis that the ER's may have different biological functions.

The discovery of ERbeta suggests the existence of two previously unrecognized pathways of estrogen signalling; via the ERbeta subtype in tissues exclusively expressing this subtype and via the formation of heterodimers in tissues expressing both ER subtypes. The existence of two ER subtypes, their differential expression pattern, and different actions on certain response elements could provide explanations for the striking species-, cell-, and promoter-specific actions of estrogens and antiestrogens.3 Both estrogen receptors appear to be involved in a multitude of regulatory events. Estrogen receptor alpha appears to play a major role in the regulation of reproductive events and estrogen receptor alpha knockout female mice are completely infertile. Estrogen receptor beta knockout females have severe but incomplete infertility. Both receptors appear to be of essence for the cardiovascular system.4

Five isoforms of the hER beta gene, designated hER beta 1-5 have been identified. The hER beta isoform mRNAs displayed a differential pattern of expression in human tissues and in tumor cell lines by RT-PCR. Further characterization of the three full length isoforms, hER beta 1-3, by in vitro band shift studies indicated that the isoforms were able to form DNA-binding homodimers and heterodimers with each other and with the ER alpha subtype.5

Reagents
Anti-Estrogen-Receptor-β (ER β) is supplied as the IgG fraction of antiserum in 0.07 M Tris-glycine, pH 7.4, containing 0.105M NaCl, 30% glycerol and 0.035% sodium azide.

Protein concentration is approximately 0.7 mg/ml.
Precautions and Disclaimer
The product is for laboratory use only.
It may NOT be used FOR in vitro diagnostics.

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability
Store at 0 °C to −20 °C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Immunoprecipitation
1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1mg/ml total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P 3813).
2. Add 5 µg of Anti-ER β to 0.5 – 1 mg cell lysate.
3. Gently rock the reaction mixture at 4 °C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A -Agarose beads (50 : 1 packed beads) (Sigma Product No. P 2545).
5. Gently rock reaction mixture at 4 °C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Immunohistochemistry
A. Deparaffinize and rehydrate as follows
   3X in xylene for 5 min.
   2X in 100% ethanol for 5 min.
   2X in 95% ethanol for 5 min.
   1X in 80% ethanol for 5 min.
B. High temperature antigen recovery
1. Place sections in a Coplin jar with dilute antigen retrieval solution of choice (0.01M citric acid, pH 6).
2. Place Coplin jar containing slides in vessel filled with water and microwave on high for 2-3 minutes (700 watt oven).
3. Check level of retrieval solution, allow to cool for 2-3 minutes and repeat steps 3, 4 four times (depending on tissue).
4. Remove Coplin jar containing sections and allow to cool for 20 minutes at room temperature.
5. Rinse sections in deionized water, 2 X for 5 minutes each.
6. Cover slides with PBS + 2% hydrogen peroxide for 5 min. to remove endogenous peroxidase activity.
7. Rinse slides one time for 5 minutes in PBS.
C. Blocking and Staining
1. Block all sections with 1% bovine serum albumin/ PBS (PBA) for 1 hour at room temperature.
2. Incubate sections in goat serum diluted in PBA (2%) for 30 minutes at room temperature to reduce immunologic binding of antibody.
3. Gently shake off excess antibody and cover sections with 10 µg/ml of Anti-Estrogen Receptor β diluted in PBA and incubate either at room temp for 1 hour or overnight at 4 °C. (Do not let sections touch during incubation)
4. Rinse sections two times for 5 minutes in PBS, shaking gently.
5. Gently remove excess PBS and cover sections with diluted goat anti-rabbit biotinylated antibody (in PBA) for 30-60 minutes at room temperature in a humidity chamber.
6. Rinse sections two times for 5 minutes in PBS, shaking gently.
7. Remove excess PBS and incubate for 1 hour at room temperature in an avidin-biotin detection system.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM Na3VO4, and 1mM NaF.
8. Rinse two times for 5 minutes in PBS, shaking gently.

D. Develop and counterstain
1. Incubate sections for approximately 2 minutes in peroxidase substrate solution made up immediately prior to use as follows (or use SIGMAFAST® DAB Tablets D 0426):
   • 10mg diaminobenzidine dissolved in 10ml 50mM sodium phosphate buffer, pH 7.4.
   • Add 12.5 µl 3% CoCl2/NiCl2 in deionized water.
   • Add 1.25 µl hydrogen peroxide. 
   **Note:** Special precautions should be taken when handling DAB because of its possible carcinogenic properties!
2. Rinse slides well three times for 10 minutes with deionized water.
3. Counterstain with 0.01% Light Green acidified with 0.01% acetic acid for 1-2 minutes depending on intensity of counterstain desired.
4. Rinse slides three times for 5 minutes with deionized water.

E. Dehydrate and mount as per normal procedures and mount slides with permanent mounting media.

**Product Profile**
Recommended use by immunotting: 1-2 µg/ml of the Anti-Estrogen-Receptor β will detect Estrogen Receptor β in RIPA lysates from LNCap and MCF-7 cells. Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Recommended use for IP: 5 µg will immunoprecipitate Estrogen Receptor β from 1mg of a rat ovary RIPA lysate.

Recommended use for immunohistochemistry: 10 µg/ml will detect the Estrogen Receptor β in human normal and carcinogenic breast tissue sections.

**Note:** In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

**References**