

Certificate of Analysis

ChIPAb+ Estrogen Receptor α

Catalog # 17-603

Lot # R0708G0052

Product Description: Every lot of the ChIPAb+ line of antibodies is individually validated for chromatin precipitation, in order to guarantee successful ChIP assays every time. Each antibody includes a control primer set for performance confirmation. ER α antibody is functionally validated in the precipitation of ER α associated chromatin.

The qPCR primers included flank the ER α binding site in human pS2 promoter.

Quantity: 25 assays per kit. ~4 μ L per chromatin immunoprecipitation.

(Dependent upon biological context)

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Kit Components

Anti-ER α (mouse ascite), Cat.# CS200620. 1 vial containing **100 μ L ascite**. Store at -20°C. The ER α antibody is made against aa120-170 of bovine estrogen α . It can recognize human and mouse ER α .

ChIP primer mix, Cat.# CS200600. 1 vial containing **75 μ L** of 5 μ M of each control primer specific for human pS2 promoter. Store at -20°C.

FOR: CCG GCC ATC TCT CAC TAT GAA

REV: CCT TCC CGC CAG GGT AAA TAC

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

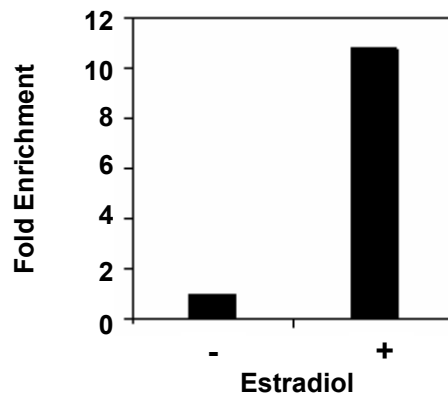
Quality Control Testing

ChIP Analysis :

Sonicated chromatin, prepared from 3×10^6 MCF7 cells that are either estrogen starved (ChIPable Chromatin Cat.# 12-689) or estradiol treated (ChIPable Chromatin Cat.# 12-690), was subjected to chromatin immunoprecipitation using 4 μ L anti-ER α (Cat.# CS200620) and the Magna ChIP™ G (Cat.# 17-611) kit Standard Protocol. Successful enrichment of ER associated DNA fragments was verified by qPCR using ChIP Primers TFF1 (pS2), (Cat.# CS200600) flanking the human TFF1 promoter that contains an ER binding site (Figure 1).

Please refer to the EZ-Magna ChIP™ G (Cat.# 17-409) or EZ-ChIP™ (Cat.# 17-371) kit protocols for experimental details.

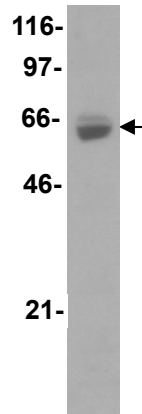
Figure 1:



Immunoblot Analysis:

MCF7cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-ER α (1:1000 dilution). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system (Figure 2). Arrow indicates Estrogen α (66 kDa).

Figure 2:



qPCR Protocol

qPCR run parameters and reagent setup are listed below. Preparing qPCR master mix is recommended for multiple PCR reactions. We suggest performing triplicate for qPCR of each individual ChIP reaction.

qPCR reagent assembly for 1 reaction:

ChIP final product	5 μ L
ddH ₂ O	6.5 μ L
Sybr-Green Master Mix	12.5 μ L
Primer mix	1 μ L
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Total	25 μ L

qPCR parameters:

Initial Denaturation 94°C 10 min	
Denature 94°C 20 sec	} 50 times
Anneal and Extension: 60°C 1 min	

References:

1. Shang, Y., et al., Cell 103: 843-52, 2000.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.