

## Certificate of Analysis

### ChIPAb+ Estrogen Receptor $\alpha$

Catalog # 17-603

Lot # DAM1480392

**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 $\alpha$  antibody is functionally validated in the precipitation of ER $\alpha$  associated chromatin.

The qPCR primers included flank the ER $\alpha$  binding site in the human pS2 promoter and produce an 80 base pair PCR product.

**Quantity:** 25 assays per kit. ~4  $\mu$ L per chromatin immunoprecipitation.

(Dependent upon biological context)

**Storage and Stability:** Stable for 1 year at -20°C from date of shipment.

### Kit Components

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

**ChIP primers TFF1 (pS2)**, Cat.# CS200600. 1 vial containing **75  $\mu$ L** of 5  $\mu$ M of each control primer specific for human TFF1 (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**

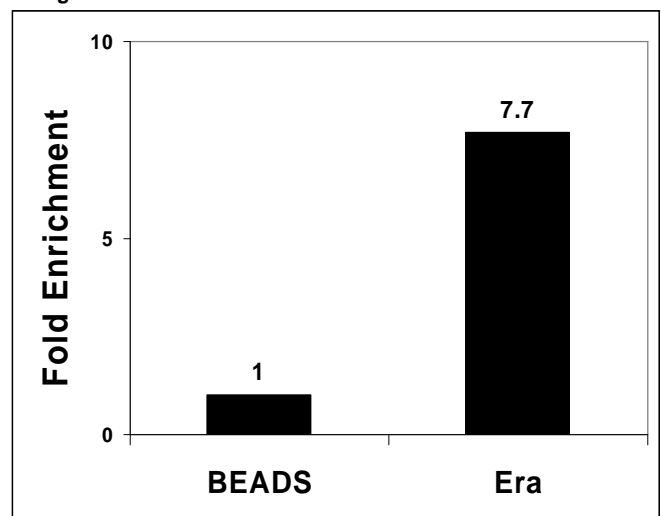
### Quality Control Testing

#### ChIP Analysis :

Sonicated chromatin prepared from  $3 \times 10^6$  MCF7 cells that were treated with  $\beta$ -estradiol (100 nM, 45 min.) was subjected to chromatin immunoprecipitation using 4  $\mu$ L anti-ER $\alpha$  (Cat.# CS200620) and beads only control. 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:



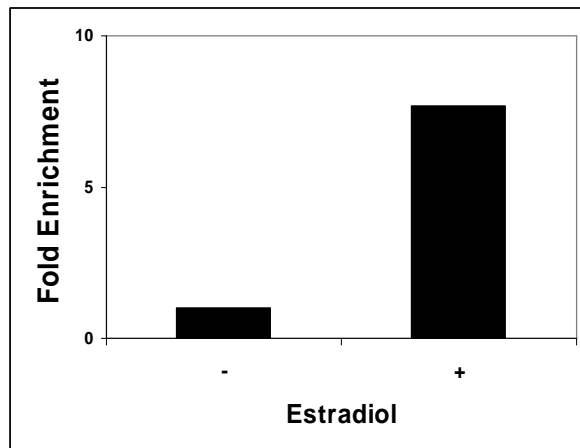
**Additional Application Testing**

ChIP Analysis :

Sonicated chromatin, prepared from  $3 \times 10^6$  MCF7 cells that are either estrogen starved or  $\beta$ -estradiol treated (100 nM, 45 min.) was subjected to chromatin immunoprecipitation using 4  $\mu$ L anti-ER $\alpha$  (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.

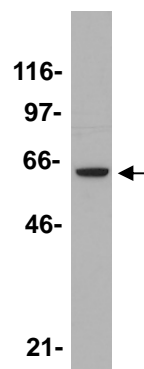
**Figure 1:**



Immunoblot Analysis:

MCF7 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-ER $\alpha$  (1:1000 dilution). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system (Figure 3). Arrow indicates Estrogen  $\alpha$  (~66 kDa).

**Figure 3:**



**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	2 $\mu$ L
ddH <sub>2</sub> O	9.5 $\mu$ L
Sybr-Green Master Mix	12.5 $\mu$ L
Primer mix	1 $\mu$ L
<b>Total</b>	<b>25 <math>\mu</math>L</b>

**qPCR parameters:**

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

} 50 times

**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.