



Technical Data Sheet

Diagenode sa
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Product name:
antibody directed against hER α

(human Estrogen Receptor alpha)

Catalog #: MAb-NRHER-050	Type: Monoclonal (IgG3 kappa)	Size: 50 μ g/ 25 μ l
Lot #: 001	Source: Mouse	Concentration: 2.0 μ g/ μ l

Description: This antibody has been raised against the NH2 terminus of the human estrogen receptor alpha using a peptide antigen (Q19-K32) conjugated to KLH. The antibody recognizes specifically hER alpha, does not bind to hER beta.

Specificity: Human: positive
Other species: not tested

Applications	Suggested dilution	References
ELISA	-	[1]
Dot blotting	Not tested	
Western blotting	7 μ g/ml	Fig 1; [1]
Gel Supershift	Not tested	
Immunochemistry	15 μ g/ml	Fig 2, [1]
Flow cytometry	Not tested	
Immunoprecipitation	-	[1]
ChIP	5 μ g per IP	Fig 3; [1]

Format: In solution in PBS 1x containing 0.01% stabilizing agent. The antibody has been purified by ammonium sulphate precipitation, followed by dialysis against PBS 1x.

Storage: Store at -20°C. Do not freeze-thaw.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

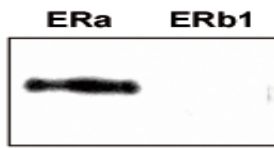
References:

[1]. Dimolea E., Pechtelidou A., Mitsiou D.J., Papalexi E., Karandrea D., Petrou C., Florentin I., Magafa V., Kitraki E., Tiniakos D.G., Kordopatis P. and Alexis M.N. *Production, characterization and applications of new specific monoclonal and polyclonal antibodies to estrogen receptor- α and - β* . In preparation.

Availability date: February 15, 2007. Last data sheet update: March 12, 2007

Lot #: 001: clone #:mAN1/ purification day: 01/16/2007

Figure 1:

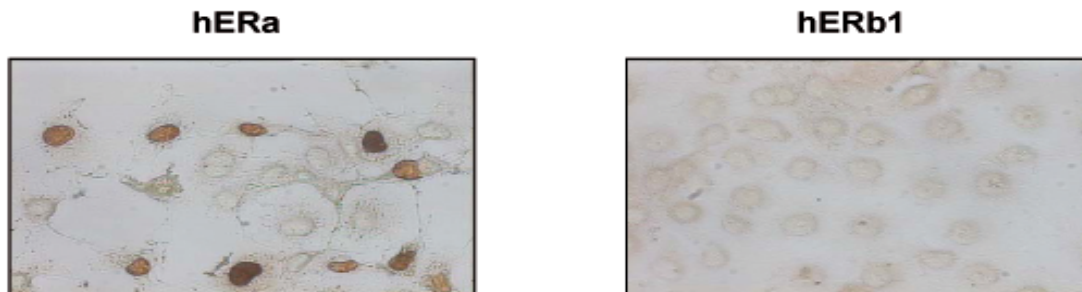


Analysis by Western blot of ER using the antibody anti-hERα from Diagenode

Specificity of the antibody is assessed by Western blot using recombinant ER alpha (right side) and recombinant ER beta (left side), 100 fmol each per well.

The monoclonal antibody directed against ER alpha (clone #:mAN1) from Diagenode is used at 7 µg/ml. The antibody is clearly recognizing the ER alpha isoform and not ER beta. (Note that the antibody is not recommended for use in WB for detection of endogenous low levels of ER expression.)

Figure 2:

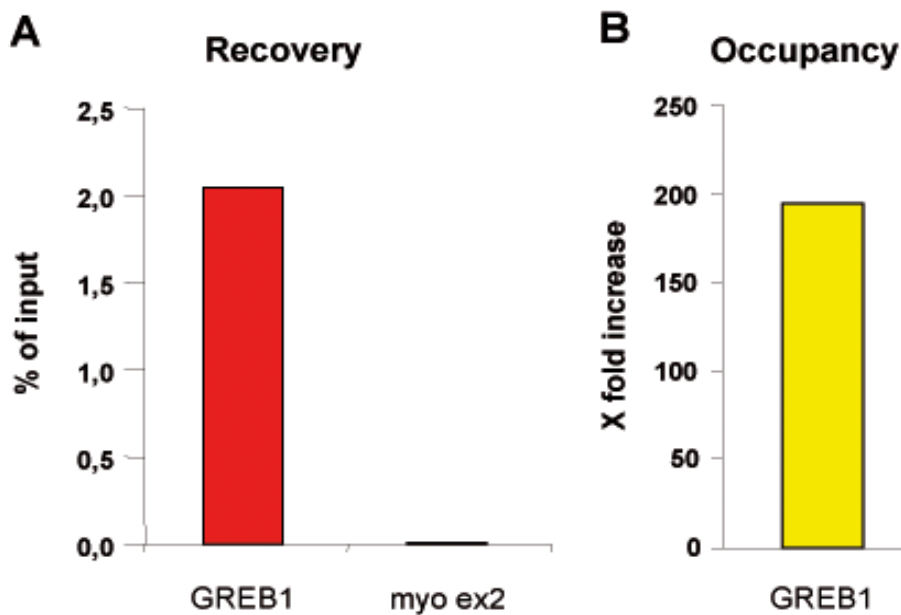


Immunocytochemistry using the monoclonal antibody anti-hERα from Diagenode

COS-7 cells transiently overexpressing human ERα (left panel) or ERβ1 (right panel) were both labeled with the antibody anti-ER alpha from Diagenode followed by biotinylated second antibody and peroxidase-labeled avidin.

The monoclonal antibody directed against ER alpha from Diagenode (clone #:mAN1) is used at 15 µg/ml. The antibody is clearly recognizing the ER alpha isoform and not ER beta.

Figure 3:



ChIP results obtained with the antibody directed against hER α from Diagenode.

ChIP assays were performed using MCF7 cells, the Diagenode monoclonal antibody directed against ER alpha (clone #:mAN1) and optimized PCR primer sets for qPCR. The cells were treated with estradiol (ER agonist) for 3 hours prior to cell harvesting.

Chromatin sheared from 3 million cells and 5 μ g of antibody anti-hER α were used per ChIP experiment. Recovery (%: ChIP/input) and occupancy (x fold: +ve/-ve) are shown here above.

In red and yellow: Recovery and occupancy of human GREB1 promoter by ER α , respectively. (Recovery of human myoglobin exon 2 (myo ex2) by ER α is shown as a negative control)

Occupancy of the human GREB1 promoter by ER α is evident based on fluorescent qPCR analysis of immunoprecipitated DNA. Controls for IP and PCR specificity include antibody directed against ER beta (data not shown) and primers for human myoglobin exon 2 (-ve control) respectively.