



Technical Data Sheet

Diagenode sa
CHU, Tour GIGA B34, 3^e étage
Avenue de l'Hôpital, 1
4000 Liège - Belgium

Product name:
antibody directed against hGR

(human Glucocorticoid Receptor)

Catalog #: MAb-NRhGR-050	Type: Monoclonal (IgG2b kappa)	Size: 50 µg/ 50 µl
Lot #: 001	Source: Mouse	Concentration: 1.0 µg/µl

Description: This antibody has been raised against the aa 304-428 of human glucocorticoid receptor (hGR) - a chimeric protein with MS2 polymerase was used-.

Specificity: Human: positive
Other species: mouse and rat, others not tested

Applications	Suggested dilution	References
ELISA	0.5 µg/ml	Fig 1
Dot blotting	Not tested	
Western blotting	1 µg/ml	Fig 2; [1]
Gel Supershift	5 µg/ml	
Immunochemistry	2.5 µg/ml	Fig 3, 4, [2,3]
Flow cytometry	0.5 µg/ml	
Immunoprecipitation	5 µg of antibody per 5 million HeLa cells/100 µl	Fig 5
ChIP (ChIP-on-chip)	5 µg per IP	Fig 6

Format: In solution in PBS 1x containing 0.01% stabilizing agent and 0.1% BSA. 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.

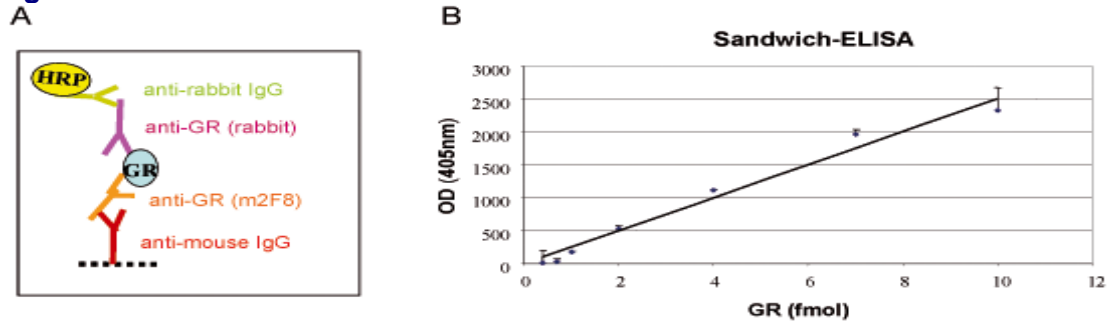
References:

- [1]. Siriani D., Mitsiou D.J. & Alexis M.N. 2005 *J Steroid Biochem Mol Biol* 94:93-101.
- [2]. Kitraki E., Kremmyda O., Youlatos D., Alexis M.N. & Kittas C. 2004 *Neuroscience* 125:47-55.
- [3]. Siriani D., Mitsiou D.J. & Alexis M.N. 2003 *J Steroid Biochem Mol Biol* 84:171-80.

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

Lot #: 001: clone #:m2F8/ purification day: 01/16/2007

Figure 1:



h-GR Sandwich ELISA

Specificity of the monoclonal antibody directed against hGR from Diagenode was assessed by sandwich Enzyme-Linked Immunosorbent Assay (ELISA). **A/** Schematic representation of the sandwich ELISA, the monoclonal antibody anti-hGR (clone #:m2F8) is used at 0.5 µg/ml. **B/** As increasing amounts of recombinant hGR are used in the ELISA, the ELISA signal is proportionally increasing.

Figure 2:

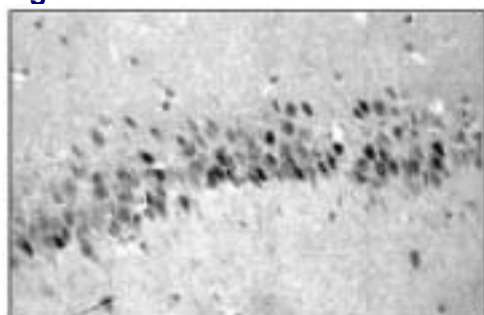


Analysis by Western blot of cell extracts using the antibody anti-hGR from Diagenode

A/ Analysis by Western blot of extracts from HeLa cells containing the indicated amounts of GR (from 30 to 240 fmol) and from 5x 10⁶ Raji cells and 5x 10⁶ Molt cells using the MAb directed against human GR from Diagenode.

B/ 300,000 HeLa cells were also analyzed by Western blot using the MAb anti-GR from Diagenode. The monoclonal antibody directed against hGR from Diagenode (clone #:m2F8) is used at 1 µg/ml.

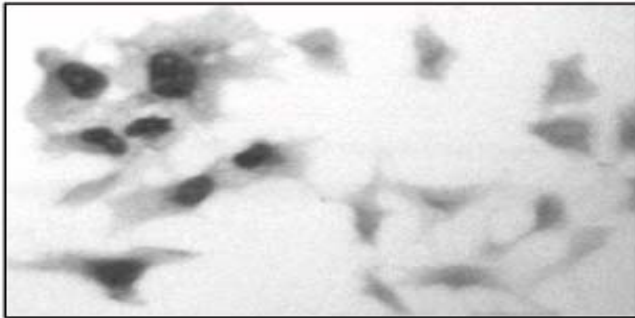
Figure 3:



Immunohistochemistry using the monoclonal antibody anti-hGR from Diagenode

Here we show GR immunoreactivity in rat CA1 neurons of hippocampus using the monoclonal antibody directed against GR from Diagenode (clone #:m2F8) used at 2.5 µg/ml.

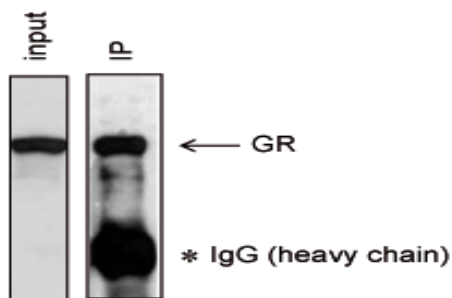
Figure 4:



Immunocytochemistry using the monoclonal antibody anti-hGR from Diagenode

COS-7 cells transiently overexpressing human GR were labeled with the antibody anti-GR from Diagenode followed by biotinylated second antibody and peroxidase-labeled avidin. The monoclonal antibody directed against GR from Diagenode (clone #:m2F8) is used at 2.5 µg/ml.

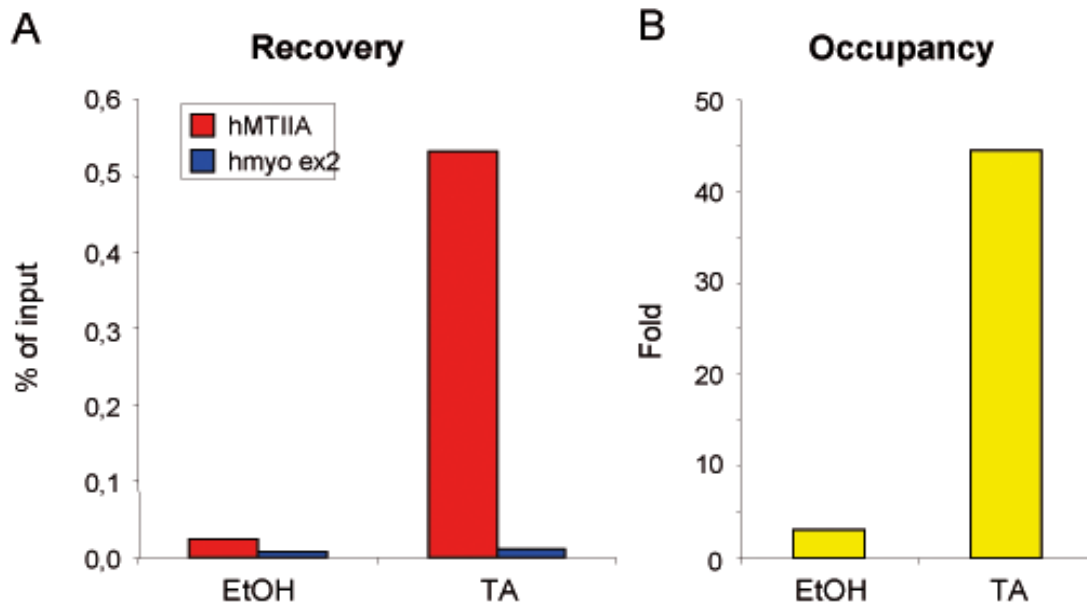
Figure 5:



Immunoprecipitation using the antibody anti-hGR from Diagenode

The immunoprecipitation (IP) of the glucocorticoid receptor from HeLa cell extracts is performed using the monoclonal antibody directed against GR from Diagenode (clone #:m2F8) as follows: 5 µg of antibody per 5 million HeLa cells in 100 µl IP reaction solution. The IP is followed by Western blot analysis with the same antibody.

Figure 6:



ChIP results obtained with the antibody directed against hGR from Diagenode.

ChIP assays were performed using HeLa cells, the Diagenode monoclonal antibody directed against GR (clone #:m2F8) and optimized PCR primer sets for qPCR. The cells were treated either with ethanol (EtOH) or triamcinolone acetonide (TA) for 4 hours prior to cell harvesting.

Chromatin sheared from 3 million cells and 5 µg of antibody anti-hGR 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 metallothionein promoter (hMTIIA) by GR, respectively.

In blue: Recovery of human myoglobin exon 2 (hmyo ex2) by GR (as a negative control)

Occupancy of the human metallothionein IIA promoter by GR is evident based on fluorescent qPCR analysis of immunoprecipitated DNA. Controls for IP and PCR specificity include primers for human myoglobin exon 2 (blue, -ve control), as well as ethanol treated cells.