# Neurofilament, Heavy chain Antibody (3G3)

## Product Data Sheet

### Tested Species Reactivity vs. Published Species Reactivity

<table>
<thead>
<tr>
<th>Tested Species</th>
<th>Published Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (Hu)</td>
<td>Drosophila (Dm)</td>
</tr>
<tr>
<td>Rat (Rt)</td>
<td>Human (Hu)</td>
</tr>
<tr>
<td></td>
<td>Mouse (Ms)</td>
</tr>
<tr>
<td>Rabbit (Rb)</td>
<td>Rat (Rt)</td>
</tr>
</tbody>
</table>

### Tested Applications

<table>
<thead>
<tr>
<th>Application</th>
<th>Dilution *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Blot (WB)</td>
<td>1:100-1:1000</td>
</tr>
<tr>
<td>Immunofluorescence (IF)</td>
<td>5 µg/ml</td>
</tr>
<tr>
<td>Immunohistochemistry (Paraffin) (IHC (P))</td>
<td>1:10-1:100</td>
</tr>
</tbody>
</table>

### Published Applications

<table>
<thead>
<tr>
<th>Application</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Blot (WB)</td>
<td>See publications</td>
</tr>
<tr>
<td>Immunocytochemistry (ICC)</td>
<td>See publications</td>
</tr>
<tr>
<td>Immunoprecipitation (IP)</td>
<td>See publications</td>
</tr>
</tbody>
</table>

* Suggested working dilutions are given as a guide only. It is recommended that the user titrates the product for use in their own experiment using appropriate negative and positive controls.

### Details

- **Catalog Number:** MA1-2012
- **Size:** 100 µg
- **Class:** Monoclonal
- **Type:** Antibody
- **Clone:** 3G3
- **Host / Isotype:** Mouse
- **Immunogen:** Synthetic peptide corresponding to 226 amino acids of the C-terminus of neurofilament, heavy chain.

### Form Information

- **Form:** Liquid
- **Concentration:** 1mg/ml
- **Purification:** purified
- **Storage Buffer:** PBS with 1mg/ml BSA
- **Preservative:** 0.05% sodium azide
- **Storage Conditions:** -20° C, Avoid Freeze/Thaw Cycles

### General Information

MA1-2012 detects the neurofilament, heavy chain in human and rat samples. MA1-2012 has been successfully used in Western blot, immunohistochemistry and immunofluorescence procedures. By Western blot, this antibody detects a ~200 kDa protein representing the neurofilament, heavy chain in rat brain microsome. In immunohistochemistry procedures, MA1-2012 recognizes the neurofilament, heavy chain in rat hippocampal neurons.

The MA1-2012 immunogen is a synthetic peptide corresponding to 226 amino acids of the C-terminus of neurofilament, heavy chain.

Like most other intermediate filament proteins (IFPs), the expression of the different neuronal IFPs is both tissue-specific and developmentally regulated. The neurofilament (NF) triplet proteins (~70, 160, and 200 kDa) occur in both the central and peripheral nervous system and are normally restricted to neurons. The 70 kDa NF-protein can self-assemble into a filamentous structure, whereas the 160 kDa and 200 kDa NF-proteins require the presence of the 70 kDa NF-protein to co-assemble.

### Notes

- This product is for in vitro experimental use only. Not for resale without express authorization.
- This warranty does not extend to anyone other than Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.
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- Questions or comments? Contact us at Pierce Biotechnology, PO Box 117, 3747 N. Meridian Road, Rockford, IL 61105 USA, (800) 874-3723, (815) 968-0747, (815) 968-7316 fax, www.thermo.com/pierce.
Western blot analysis of Neurofilament, Heavy chain was performed by loading 25 µg of SH-SY5Y (lane 1) and rat brain (lane 2) lysates onto an SDS polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked at 4°C overnight. The membrane was probed with a Neurofilament, Heavy chain monoclonal antibody (Product # MA1-2012) at a dilution of 1:500 overnight at 4°C, washed in TBST, and probed with an HRP-conjugated secondary antibody for 1 hr at room temperature in the dark. Chemiluminescent detection was performed using Pierce ECL Plus Western Blotting Substrate (Product # 32132). Results show a band at ~200kDa.

Immunofluorescence with anti-Neurofilament, Heavy chain Monoclonal Antibody [3G3] (MA1-2012)

Immunofluorescence of neurofilament, heavy chain in rat cerebral cortex cultures in green.

Immunohistochemistry (Paraffin) with anti-Neurofilament, Heavy chain Monoclonal Antibody [3G3] (MA1-2012)

Immunohistochemistry analysis of Neurofilament, Heavy chain showing staining in the cytoplasm and axons of formalin-fixed, paraffin-embedded human brain tissue (B) and magnified section (C) compared with an isotype control (A). To expose target proteins, antigen retrieval was performed using HEIR with a buffer (pH 6.2). Tissues were probed with a Neurofilament, Heavy chain monoclonal antibody (Product # MA1-2012) for 60 minutes at a dilution of 2µg/ml and detection was performed using an HRP-conjugated detection system for 30 minutes followed by DAB staining.

Immunohistochemistry (Paraffin) with anti-Neurofilament, Heavy chain Monoclonal Antibody [3G3] (MA1-2012)

Immunohistochemistry analysis of Neurofilament Heavy Chain showing staining in the cytoplasm and nucleus of paraffin-embedded rat brain tissue (right) compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a Neurofilament Heavy Chain monoclonal antibody (Product # MA1-2012) diluted in 3% BSA-PBS at a dilution of 1:20 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.

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### PubMed References for Neurofilament, Heavy chain Antibody (3G3)

#### 4 Western Blot References

<table>
<thead>
<tr>
<th>Species / Dilution</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ms / Not Cited</td>
<td><strong>MA1-2012</strong> was used in immunoprecipitation and western blot to investigate the protein components of the HSP90 complex.</td>
</tr>
</tbody>
</table>
|                    | "Evidence that the 90-kDa heat shock protein (HSP90) exists in cytosol in heteromeric complexes containing HSP70 and three other proteins with Mr of 63,000, 56,000, and 50,000."  
|                    | Author(s): Perdew GH, Whitelaw ML  
|                    | Number of Citations: 21  

**Ms / Not Cited**

**MA1-2012** was used in immunoprecipitation and western blot to investigate the role of self-sufficient protein folding structure, a "foldosome" during assembly of the glucocorticoid receptor into a functional heterocomplex with heat shock protein 90.


"All the factors required for assembly of the glucocorticoid receptor into a functional heterocomplex with heat shock protein 90 are preassociated in a self-sufficient protein folding structure, a "foldosome"."  
Author(s): Hutchison KA, Dittmar KD, Pratt WB  
Number of Citations: 12  

| Rb / 1:2,000 | **MA1-2012** was used in immunoprecipitation and western blot to study the function of p23 during the process of receptor activation by hsp foldosome  
| "The 23-kDa acidic protein in reticulocyte lysate is the weakly bound component of the hsp foldosome that is required for assembly of the glucocorticoid receptor into a functional heterocomplex with hsp90."  
| Author(s): Hutchison KA, Stancato LF, Owens-Grillo JK, Johnson JL, Krishna P, Toft DO, Pratt WB  
| Number of Citations: 21  

| Rt / Not Cited | **MA1-2012** was used in immunocytochemistry and western blot to study the subcellular localization of alpha internexin and neurofilament triplet proteins  
| "Compartmentation of alpha-internexin and neurofilament triplet proteins in cultured hippocampal neurons."  
| Author(s): Benson DL, Mandell JW, Shaw G, Banker G  
| Number of Citations: 2  

### 1 Immunocytochemistry Reference

<table>
<thead>
<tr>
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</tr>
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</table>
| Rt / 1:200 | **MA1-2012** was used in immunocytochemistry and western blot to study the subcellular localization of alpha internexin and neurofilament triplet proteins  
| "Compartmentation of alpha-internexin and neurofilament triplet proteins in cultured hippocampal neurons."  
| Author(s): Benson DL, Mandell JW, Shaw G, Banker G  
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### 13 Immunoprecipitation References

<table>
<thead>
<tr>
<th>Species / Dilution</th>
<th>Summary</th>
</tr>
</thead>
</table>
| Dm / Not Cited | **MA1-2012** was used in immunoprecipitation to study the interaction between Sim and hsp90  
| "The basic helix-loop-helix/PAS factor Sim is associated with hsp90. Implications for regulation by interaction with partner factors."  
| Author(s): McGuire J, Coumailleau P, Whitelaw ML, Gustafsson JA, Poellinger L  
| Number of Citations: 9  
MA1-2012 was used in immunoprecipitation to investigate the role of a tyrosine kinase-dependent pathway during ligand-dependent activation of the dioxin receptor in human keratinocytes

“A tyrosine kinase-dependent pathway regulates ligand-dependent activation of the dioxin receptor in human keratinocytes.”
Author(s): Gradin K, Whitelaw ML, Toftgård R, Poellinger L, Berghard A
Number of Citations: 3

MA1-2012 was used in immunoprecipitation and western blot to investigate the protein components of the HSP90 complex

“Evidence that the 90-kDa heat shock protein (HSP90) exists in cytosol in heteromeric complexes containing HSP70 and three other proteins with Mr of 63,000, 56,000, and 50,000.”
Author(s): Perdew GH, Whitelaw ML
Number of Citations: 21

MA1-2012 was used in immunoprecipitation to investigate the functional domains of mouse aryl hydrocarbon receptor

“Identification of functional domains of the aryl hydrocarbon receptor.”
Author(s): Fukunaga BN, Probst MR, Reisz-Porszasz S, Hankinson O
Number of Citations: 25

MA1-2012 was used in immunoprecipitation to detect the interaction between the dioxin receptor and Hsp90

“Definition of a minimal domain of the dioxin receptor that is associated with Hsp90 and maintains wild type ligand binding affinity and specificity.”
Author(s): Coumailleraud P, Poellinger L, Gustafsson JA, Whitelaw ML
Number of Citations: 22

MA1-2012 was used in immunoprecipitation and western blot to investigate the role of self-sufficient protein folding structure, a “foldosome” during assembly of the glucocorticoid receptor into a functional heterocomplex with heat shock protein 90

“All of the factors required for assembly of the glucocorticoid receptor into a functional heterocomplex with heat shock protein 90 are preassociated in a self-sufficient protein folding structure, a “foldosome”.”
Author(s): Hutchison KA, Dittmar KD, Pratt WB
Number of Citations: 12

MA1-2012 was used in immunoprecipitation to investigate the role of Arnt for release of hsp90 from the dioxin receptor in the presence of dioxin

“A cellular factor stimulates ligand-dependent release of hsp90 from the basic helix-loop-helix dioxin receptor.”
Author(s): McGuire J, Whitelaw ML, Pongratz I, Gustafsson JA, Poellinger L
Number of Citations: 18

MA1-2012 was used in immunoprecipitation to study the folding of hormone binding domain of glucocorticoid receptor in vitro using reconstituted receptor-hsp90 heterocomplex assembly system with purified components

“Folding of the glucocorticoid receptor by the reconstituted Hsp90-based chaperone machinery. The initial hsp90,p60,hsp70-dependent step is sufficient for creating the steroid binding conformation.”
Author(s): Dittmar KD, Pratt WB
Number of Citations: 26

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Pierce Biotechnology
PO Box 117
3747 N. Meridian Road
Rockford, IL 61105 USA
(800) 874-3723
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MA1-2012 was used in immunoprecipitation and western blot to study the function of p23 during the process of receptor activation by hsp foldosome

"The 23-kDa acidic protein in reticulocyte lysate is the weakly bound component of the hsp foldosome that is required for assembly of the glucocorticoid receptor into a functional heterocomplex with hsp90."  
Author(s): Hutchison KA, Stancato LF, Owens-Grillo JK, Johnson JL, Krishna P, Toft DO, Pratt WB  
Number of Citations: 21  

MA1-2012 was used in immunoprecipitation to study the interaction of different protein components of the heat shock protein heterocomplex

"Characterization of the protein-protein interactions determining the heat shock protein (hsp90.hsp70.hsp56) heterocomplex."  
Author(s): Czar MJ, Owens-Grillo JK, Dittmar KD, Hutchison KA, Zacharek AM, Leach KL, Deibel MR Jr, Pratt WB  
Number of Citations: 11  

MA1-2012 was used in immunoprecipitation to study the mechanisms of immunophilin-mediated protein targeting

"A model of protein targeting mediated by immunophilins and other proteins that bind to hsp90 via tetratricopeptide repeat domains."  
Author(s): Owens-Grillo JK, Czar MJ, Hutchison KA, Hoffmann K, Perdew GH, Pratt WB  
Number of Citations: 26  

MA1-2012 was used in immunoprecipitation to show HIF-1α is associated with the molecular chaperone hsp90 in vitro

"Functional interference between hypoxia and dioxin signal transduction pathways: competition for recruitment of the Arnt transcription factor."  
Author(s): Gradin K, McGuire J, Wenger RH, Kvietikova I, flitelaw ML, Toftgård R, Tora L, Gassmann M, Poellinger L  
Number of Citations: 38  

MA1-2012 was used in immunoprecipitation to identify PP5 as an important component of glucocorticoid receptor-hsp90 complexes and investigate its functional properties

"Protein phosphatase 5 is a major component of glucocorticoid receptor.hsp90 complexes with properties of an FK506-binding immunophilin."  
Author(s): Silverstein AM, Galigniana MD, Chen MS, Owens-Grillo JK, Chinkers M, Pratt WB  
Number of Citations: 33  