

PRODUCT DATA SHEET

Revised: 12 January 2004
Printed: 28 January 2009; page 1 of 1



Product name:	Mouse monoclonal antibody to α-fodrin (αII-spectrin)				
Catalogue number:	FG 6090	Batch number:	Z04769	Expiry date:	12 months from receipt

Introduction:

Fodrin, also referred to as non-erythroid (α II-; brain) spectrin, is a tetrameric ($\alpha\gamma$)₂ actin-binding, fibrous protein, widely distributed in vertebrates, which forms part of the sub-membranous cytoskeleton within many cell types including neurons, and is particularly abundant with axons. The α -subunits of fodrins and spectrin are highly conserved phylogenetically, with the exception of human α -fodrin¹, which shares only 55-59% homology with erythroid-specific α -spectrins. The β -subunits of spectrin (and γ -subunits of fodrins) are species specific.

The interleukin-1 converting enzyme (ICE) family of proteases has been implicated as important effectors of the apoptotic pathway, perhaps acting hierarchically in a protease cascade². Neuronal fodrin is known to be cleaved by calpain following ischaemic insult and it has been proposed that calpain and an unidentified protease play a rôle in the onset of neuronal death following transient forebrain ischaemia³. Recently, an ICE-like protease has been implicated in the early cleavage of fodrin, producing a 150kDa fragment, proximal to CPP32 in fas-induced² and C₂-ceramide-mediated³ apoptosis. A cleavage product of α -fodrin has been proposed as a candidate autoantigen in primary Sjögren's syndrome⁵ and α -fodrin has been shown to be the source of a so-called 'inhibitory protein factor' family, members of which have been shown to inhibit both GABA and ATP-dependent glutamate uptake into purified synaptic vesicles⁶.

Product information:

The hybridoma (clone AA6) secreting the antibody to α -fodrin was generated by immunising Balb/c mice with chicken blood cell membranes following hypotonic lysis and mechanical enucleation. The antibody has been extensively characterised by Western blotting and immunohistochemistry.

Vial contains an immunoglobulin preparation, partially purified from exhausted supernatant, suspended in phosphate-buffered saline containing 0.01M sodium azide at a concentration of 100 μ g/mL. The antibody is an IgG₁.

This antibody reacts with the 240/280kDa α -fodrin molecule from all mammalian non-erythroid cells, and with chicken α -spectrin in Western blotting* and immunocytochemical applications at dilutions of 1:100-1:500⁷ or greater⁸ under optimised conditions. Following neuronal injury in rats, calpain/caspase-3 cleavage products of 150/145/120kDa size are observed on Western blots^{8,9} (see Figure).

For immunocytochemistry the use of methanol-fixed or cold acetone-fixed fresh frozen cryostat or cell culture

preparations is recommended, although formaldehyde-containing fixatives may also be used. The antibody does not appear to be suitable for use on de-paraffinised tissue as the reactive epitope(s) on α -fodrin becomes compromised by the necessary conditions of embedment.

NOTES: Optimal dilutions must be determined by experimentation. *Indirect immunoperoxidase procedure with overnight incubation in primary antibody at 4°C. Detection using ECL procedure (Amersham International plc), exposure for 1min. The antibody is known to react with rat and human α -fodrin and is thought to exhibit reasonably broad species reactivity.



Rat primary septo-hippocampal co-cultures (astrocytes and neurons cultured from day E18 rat embryos) were challenged with the *pro*-apoptotic compound staurosporine or with vehicle (DMSO) for times indicated above. Protein (40 μ g) was separated by SDS-PAGE, transferred to PVDF membrane and probed with FG6090. The caspase-3 cleaved 120-kDa fragment of α II-spectrin was detected at a dilution of 1:4000.

Western blot provided by courtesy of Dr Brian Pike, University of Florida.

Storage and use:

Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes (not less than 10 μ L) prior to freezing if appropriate. The use of high quality 'antiserum-grade' plastic or glass vials is recommended. Store diluted antibody at 2-4°C (do not freeze) and use within 1 month.

Dilute to working strength with 50mM Tris-HCl buffer (pH 7.6) containing 1.5% sodium chloride and 1% normal goat serum (if a goat anti-mouse IgG linker antibody is to be used).

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References:

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- 3 Craighead, M., Pole, J. and Waters, C. Caspases mediate C2-ceramide induced apoptosis of the human oligodendroglial cell line, MO3.13. *Neurosci. Lett.*, **278**: 125-128, 2000 [CITATION].
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- 8 Pike, B.R. *et al.* Accumulation of non-erythroid α II-spectrin and calpain-cleaved α II-spectrin breakdown products in cerebrospinal fluid after traumatic brain injury in rats. *J. Neurochem.*, **78**: 1297-1306, 2001 [CITATION].
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Other citations to the use of FG 6090:

- Blomgren, K. *et al.* Synergistic activation of caspase-3 by mu-calpain after neonatal hypoxia-ischaemia. *J. Biol. Chem.*, **276**: 10191-10198, 2001.
- Ishimaru, N. *et al.* Possible role of organ-specific autoantigen for Fas ligand-mediated activation-induced cell death in murine Sjogren's syndrome. *J. Immunol.*, **167**: 6031-6037, 2001.
- Nath, R. *et al.* Development and characterization of antibodies specific to caspase-3-produced alpha II-spectrin 120kDa breakdown product: Marker for neuronal apoptosis. *Neurochem. International*, **37**: 351-361, 2000.