

PRODUCT DATA SHEET

Revised: 14 February 2004
Printed: 30 January 2009; page 1 of 2



Product name(s):	Rabbit polyclonal antiserum to 7B2
-------------------------	------------------------------------

Catalogue number:	SA 1100	Batch number:	Z06035	Expiry date:	12 months from receipt
--------------------------	---------	----------------------	--------	---------------------	------------------------

Product information:
<p>Known variously as APPG, secretogranin V and neuroendocrine protein I, 7B2 was originally isolated from porcine pituitary in 1983¹. It soon became evident that 7B2-like immunoreactants were present in many cell types from a variety of species²⁻⁴, and it is now recognised to be widely distributed in neural and endocrine tissues.</p> <p>In the early 1990s full length (27kDa) 7B2 was shown to be a potent inhibitor of prohormone convertase PC2 activity⁵⁻⁷, a subtilisin-like enzyme responsible for the proteolytic cleavage of many neuroendocrine precursor molecules, including proenkephalin, POMC and gastrin-17⁸. mRNA distribution analysis⁹ revealed that in adult rat brain expression of 7B2 mRNA was <i>pan</i>-neuronal, whereas PC2 mRNA was exclusively neuronal, but more restricted. PC2+/7B2- cells were not encountered in the adult, but were common in developing rat brain. These findings suggest that 7B2 may have intracellular functions other than PC2 maturation in certain cells, although some recent work shows a discordancy between mRNA expression and enzymic activity¹⁰.</p> <p>The mechanism for PC2 maturation appears to involve <i>pro</i>7B2 binding to inactive <i>pro</i>PC2 <i>via</i> its polyproline motif (¹¹⁶PPNCP¹²¹) in the endoplasmic reticulum and chaperoning <i>pro</i>PC2 to a later secretory pathway compartment where maturation of PC2 proceeds once <i>pro</i>-7B2 is proteolytically processed by a furin-like convertase¹¹⁻¹³ into a 21kDa fragment and a C-terminal peptide. It is the C-terminal peptide that potently inhibits maturation of <i>pro</i>PC2 until the complex is subjected to a decreasing pH gradient along the secretory pathway¹⁴. It seems likely that mature PC2 then cleaves the CT peptide at its internal lys-lys site¹³. 7B2 knockout mice develop a severe Cushing's-like phenotype¹⁵ and exhibit multiple metabolic and hormonal abnormalities¹⁶, indicating that 7B2 is required for activation of PC2 <i>in vivo</i>. Conversely, PC2-null mice appear to be viable¹⁵. Readers are referred to a review by Mbikay and others for more information on 7B2¹⁷.</p>

Product information:
<p>The rabbit polyclonal antiserum was raised to a synthetic peptide corresponding to residues 23-39 of porcine 7B2 (<i>hpro</i>7B2[49-65])¹⁸ conjugated to bovine serum albumin using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide. The amino acid sequence used (EQLGIARPRVEYPAHQA) is conserved in human, pig, rat and mouse 7B2 proteins. Vial contains a partially purified immunoglobulin preparation containing 0.01M sodium azide.</p> <p>Application data 7B2; alternative names: secretogranin V, neuroendocrine protein I; GenBank accession number: 23830842; length: 212 amino acids.</p> <p>Immunoblotting – The antibody detects a single band on western blots at 25-27kDa in rat brain, rat pituitary and PC12 cell lysates*. The band is wholly abolished by preincubation with the immunising peptide (see product number SP 9102).</p> <p>Immunoprecipitation - This antibody has not been characterised for use in immunoprecipitation.</p> <p>Immunohistochemistry - The antibody has been tested on de-waxed tissue sections of human endocrine pancreas and rat anterior pituitary. Neutral buffered formaldehyde is recommended for fixation, but other standard cross-linking agents have been used successfully. A dilution range of 1:500-1:2000 is recommended^{19**}. A standard protocol should be followed in combination with sensitive detection methods, such as ABC-peroxidase (Vector-Elite).</p> <p>NOTES: *Indirect immunoperoxidase procedure with overnight incubation in primary antibody at 4°C, 1:500 dilution, chemiluminescence detected using ECL reagents (Amersham). **Optimal dilutions must be determined by experimentation.</p>

Storage and use:
<p>Store unopened vial at -20°C until required for use. AVOID REPEATED FREEZE-THAW CYCLES. Aliquot undiluted antibody into smaller volumes (not less than 10µL) 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.</p> <p>Dilute to working strength with 50mM phosphate buffered saline (pH 7.2) containing 1.5% sodium chloride and 1% normal goat serum (if a goat anti-rabbit IgG linker antibody is to be used).</p>

Product name(s):	Rabbit polyclonal antiserum to 7B2
-------------------------	------------------------------------

Catalogue number:	SA 1100
--------------------------	---------

References:

1. Hsi, K.L. *et al.* Isolation and NH₂-terminal sequence of a novel porcine anterior pituitary polypeptide. Homology to proinsulin, secretin and Rous sarcoma virus transforming protein TVFV60. *FEBS Letters*, **147**: 261-266, 1982.
2. Suzuki, H. *et al.* Ontogeny of a novel pituitary protein (7B2) in the human fetal intestine. *Regul. Pep.*, **12**: 289-296, 1985.
3. Marcinkiewicz, M. *et al.* Immunocytochemical localization of a novel pituitary protein (7B2) within the rat brain and hypophysis. *J. Histochem. Cytochem.*, **33**: 1219-1226, 1985.
4. Suzuki, H. *et al.* A novel pituitary protein (7B2)-like immunoreactivity is secreted by a rat pheochromocytoma cell line (PC12). *J. Endocrinol.*, **108**: 151-155, 1986.
5. Martens, G.J. *et al.* The neuroendocrine polypeptide 7B2 is an endogenous inhibitor of prohormone convertase PC2. *Proc. Natl. Acad. Sci. USA*, **91**: 5784-5787, 1994.
6. Lindberg, I. *et al.* Enzymatic characterization of immunopurified prohormone convertase 2: Potent inhibition by a 7B2 peptide fragment. *Biochemistry*, **34**: 5486-5493, 1995.
7. Van Horsen, A.M. *et al.* Identification of the region within the neuroendocrine polypeptide 7B2 responsible for the inhibition of prohormone convertase PC2. *J. Biol. Chem.*, **270**: 14292-6, 1995.
8. Rehfeld, J.F., Lindberg, I. and Friis-Hansen, L. Progastrin processing differs in 7B2 and PC2 knockout animals: A role for 7B2 independent of action on PC2. *FEBS Lett.*, **510**: 89-93, 2002.
9. Seidel, B. *et al.* Neuroendocrine protein 7B2 is essential for proteolytic conversion and activation of proprotein convertase 2 in vivo. *DNA Cell Biol.*, **17**: 1017-1029, 1998.
10. Li, Q.L. *et al.* Prohormone convertase 2 enzymatic activity and its regulation in neuro-endocrine cells and tissues. *Regul. Pep.*, **110**: 197-205, 2003.
11. Benjannet, S. *et al.* 7B2 is a specific intracellular binding protein of the prohormone convertase PC2. *J. Neurochem.*, **64**: 2303-11, 1995.
12. Zhu, X. and Lindberg, I. 7B2 facilitates the maturation of proPC2 in neuroendocrine cells and is required for the expression of enzymatic activity. *J. Cell Biol.*, **129**: 1641-1650, 1995.
13. Zhu, X. *et al.* Internal cleavage of the inhibitory 7B2 carboxyl-terminal peptide by PC2: A potential mechanism for its inactivation. *Proc. Natl. Acad. Sci. USA*, **93**: 4919-4924, 1996.
14. Lamango, N.S. *et al.* The proteolytic maturation of prohormone convertase 2 (PC2) is a pH-driven process. *Arch. Biochem. Biophys.*, **362**: 275-282, 1999.
15. Westphal, C.H. *et al.* The neuroendocrine protein 7B2 is required for peptide hormone processing in vivo and provides a novel mechanism for pituitary Cushing's disease. *Cell*, **96**: 689-700, 1999.
16. Sarac, M.S., Zieske, A.W. and Lindberg, I. The lethal form of Cushing's in 7B2 null mice is caused by multiple metabolic and hormonal abnormalities. *Endocrinology*, **143**: 2324-2332, 2002.
17. Mbikay M., Seidah, N.G. and Chrétien, M. Neuroendocrine secretory protein 7B2: Structure, expression and functions. *Biochem. J.*, **357**: 329-342, 2001.
18. Martens, G.J. Cloning and sequence analysis of human pituitary cDNA encoding the novel polypeptide 7B2. *FEBS Lett.*, **234**: 160-164, 1988.
19. Hacker, G.W. *et al.* Multiple peptide production and presence of general neuroendocrine markers detected in 12 cases of human pheochromocytoma and in mammalian adrenal glands. *Virchows Arch. A Pathol. Anat. Histopathol.*, **412**: 399-411, 1988.