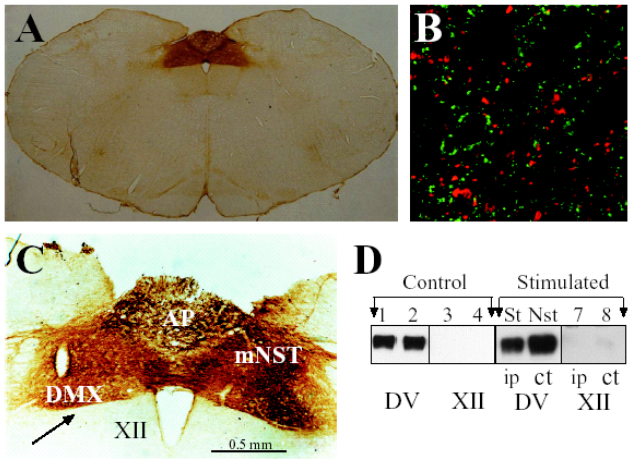
Anti PolySialic Acid-NCAM: anti-PSA NCAM AbC0019

I Product Introduction

Neural **C**ell **A**dhesion **M**olecule (NCAM) and its polysialylated form PSA-NCAM has been found to be involved in various aspects of neural and synaptic plasticity.
AbCys mouse antibody to PolySialic Acid NCAM (PSA-NCAM: catalog Number AbC0019) was produced to viable Meningococcus group B (strain 355). AbC0019 reacts with alpha 2-8 linked neuraminic acid (NeuAc-alpha 2-8) n, with n > 10.This polymer is usually termed polysialic acid (PSA). In vertebrates PSA is essentially, if not exclusively linked to NCAM, (CD56), in bacteria it is associated with capsula of meningococcus strain group B.

The monoclonal 2-2B (mouse IgM) can be used for Western Blot, Immunochemistry, Cell sorting, RIA.

Universal: AbC0019 is not species-specific and could be used in various species (including mouse).



Localization of PSA in the adult rat dorsal vagal complex (DVC) (A,B). [1]

A) Transverse section of rat caudal medulla showing the distribution of
PSA immunolabeling using mouse IgM anti-PSA monoclonal antibody (dilution 1/2000 of the ascitic fluid). (B) Confocal observation of immunofluorescence double-staining with anti-PSA (green) and anti-GAP-43 (red) antibodies showing a punctate distribution of PSA in close apposition with GAP-43 staining in the dorsal vagal complex.

Quantitative analysis of regulation of PSA expression (C,D) [1]:

(C) Enlarged section showing PSA immunoreactivity in the DVC after stimulation of the cervical vagus nerve (15 min, 30 Hz). The arrow shows the stimulated side. (D) Example of a typical western blot showing the expression of PSA in Control and in stimulated adult rat (St) in the DVC and in the Hypoglossal Nucleus (XII). DVC was separated in two halves and immunoreactivity separately detected in each of them.
Note the decrease of immunoreactivity on the stimulated side detectable both by immunohistochemistry and immunoblot with the anti-PSA antibody.

Abbreviations :
AP : Area Postrema, ST : Solitary Tract, NST : Nucleus of the Solitary Tract (mNST : medial NST),
DMX : Dorsal Motor Nucleus of the Vagus Nerve, XII : Hypoglossal Nucleus.

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II Applications

A Diagnosis Marker for medulloblastoma (neuroblastoma)

Polysialic acid (PSA) on NCAM is a carbohydrate modulating homophilic and heterophilic adhesion mediated by NCAM, is also known to be re-expressed in several human tumors, thus can be considered as an oncodevelopmental antigen.
Medulloblastomas (MBs) are highly malignant tumors that arise from the cerebellum and occur mainly in children ; they have a high frequency of metastasis through the CSF. At present, the diagnosis of meningeal spread is based both on imaging (computed tomographic scan or magnetic resonance imaging and cytologic examination). Howewer, these techniques sometime fail to detect recurrences.

PSA-NCAM levels can be measured in CSF using a double site enzyme-linked immunoadsorbant assay (ELISA). Below data in 145 samples from 14 controls and 29 patients with MB [15].

Time	PSA-NCAM/Cytology	PSA-NCAM/Imaging	PSA-NCAM/Clinical
1 month after surgery	0.37/ weak	0.89/ excellent	0.43/ average
During treatment	0.82/ excellent	0.62/ good	0.82/ excellent
After treatment	0.72/ excellent	0.54/ average	0.72/ good

Table 1 Agreement Between PSA-NCAM and Cytology, imaging, and Clinical Data at three Time Periods Following Surgery.

PSA-NCAM was never detected in control CSF. PSA-NCAM concentration medians were higher in CSF with metastatic cells or in patients showing abnormal imaging than in the corresponding normal groups ($P < .05$). The PSA-NCAM concentration median was significantly higher($P < .05$) in CSF from patients refractory to treatment or who relapsed than from patients in remission. Agreements between PSA-NCAM and clinical status and between PSA-NCAM and cytology were excellent during and after treatment. The sensitivity of PSA-NCAM test was always better than that of cytology, whereas its specificity was lower for phases that corresponded to more than 1 month following surgery. Specificity was 100% for patients refractory to treatment or with relapse.

PSA-NCAM measurement is a new biologic marker of possible use in the management of patients with MB.

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B PSA-NCAM: Marker for neurotoxicolgy

Example 1 : Nicotine Effect on the Hippocampal Plasticity [22].

Neurogenesis, which defines the production of new neurons by active proliferation of progenitor cells, is maintained in the adult dentate DG of the hippocampus, and this phenomenon seems to play an important role in learning.
Modifications of PSA-NCAM expression in mice deleted for NCAM the PSA carrier, results in morphological modifications, perturbations of synaptic plasticity and impairment of cognitive functions.
The effects of nicotine addiction on plasticity related processes in the DG has been studied in animals trained to self –administer nicotine were investigated [22].

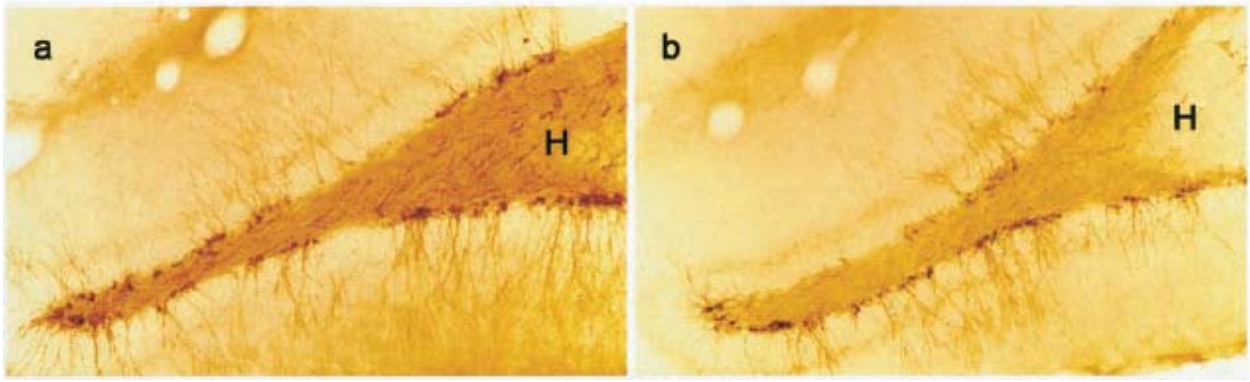

Figure 2 Illustration of PSA-NCAM labeled cells in the dentate gyrus.Microphotography of PSA-NCAM staining in a control animal (a) and in an animal self- administering 0.04mg/kg per infusion of nicotine. H, Hilus Magnification 100X

Because PSA-NCAM is expressed by newborn cells, the decrease in PSA-NCAM observed here (see b in fig. 2) results essentially from a decrease in neurogenesis.

In this study it was found that nicotine self-administration profoundly decreased the expression of PSA-NCAM and neurogenesis in the DG. In parallel, cell death was increased. PSA- NCAM can serve as a useful early marker in neurotoxicolgy.

Example 2 : PSA-NCAM and antidepressants.

Treatment with antidepressants leads to adaptive changes such as modifications of neurogenesis. PSA-NCAM is a useful marker to follow the levels of neurogenesis and can so serve as **marker to follow the efficiency of antidepressants [19-20-21].**

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C PSA-NCAM: a neuronal lineage selection marker for the early stages of ES cell differentiation [23]

In this study, *in vitro* differentiation of ES cells was induced by aggregation to embryoid bodies. Four-day-old embryoid bodies were plated and exposed to chemically defined media favoring neural differentiation . Neural precursors were further expanded in the presence of FGF2 and subsequently induced to differentiate by growth factor withdrawal.

Neural lineage selection was perform by immunopanning.
A 10-cm petri dish was covered with a biotinylated anti-mouse IgM antibody, and incubated with the **PSA-NCAM antibody.** Differentiated neural cultures withdrawn from FGF2 for 2 days were plated onto the immunopanning dish, and incubated. Following washes, panned cells were recovered, and replated in polyornithine-coated cell culture dishes.

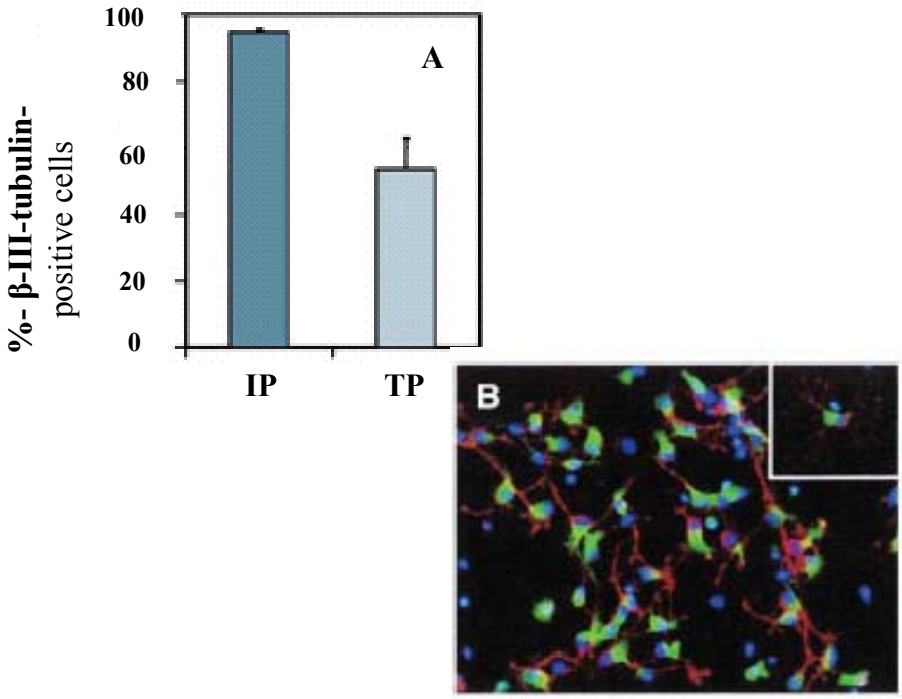

Figure 3 Enrichment of ES cell-derived neurons by PSA-NCAM immunopanning. **(A)** Following a 2-day-growth factor withdrawal, ES cell-derived neural precursors were subjected to immunopanning, yielding purities of 95.3 ± 0.9% neurons. Bars represent the mean fraction of β-III-tubulin-positive cells in the selected (IP) and nonselected (total) population (TP) 2 days after replating (SEM of three independent experiments). **(B)** PSA-NCAM-positive cells (red) double labeled with an antibody to β-III-tubulin (green) 2 days after immunopanning. Nuclei are counterstained with Hoechst (Inset: detail).

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PSA-NCAM immunopanning of precursors withdrawn from FGF2 for two days yielded highly enriched neuronal cultures. A quantification of β-III-tubulin - and PSA-NCAM-immunoreactive cells performed 2 days after immunopanning and replating showed that 99.4 % of the selected population expressed PSA-NCAM.

PSA-NCAM represent a suitable marker for high-purity lineage selection of ES cell-derived neurons.
These cells can be selected and enriched by using PSA-NCAM in immunological methods such as immunopanning, magnetic [24], and fluorescence-based selection methods.

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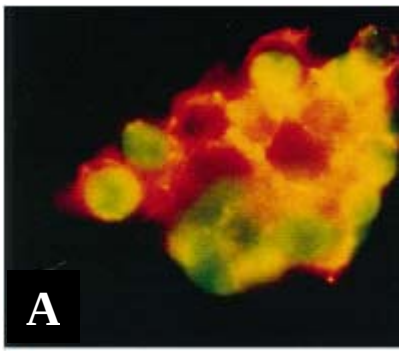
III Datasheet

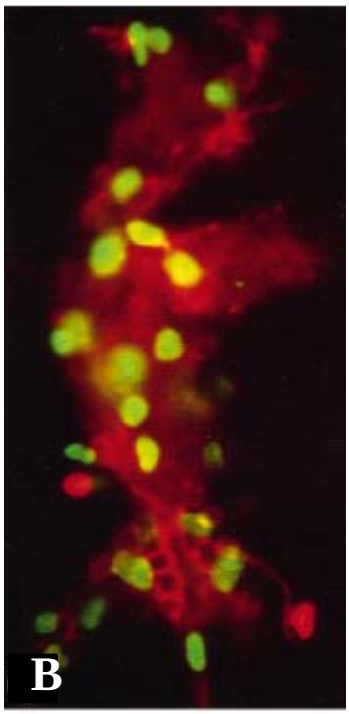
CATALOG NUMBER: AbC0019
QUANTITY: 50 µL
SPECIFICITY: Reacts with alpha 2-8 linked neuraminic acid (NeuAc-alpha 2-8) n with n >10.
IMMUNOGEN: Viable Meningococcus group B (strain 355).
ISOTYPE: IgM
CLONE: 2-2B
APPLICATIONS:
- Western blotting: 1:500-1:1,000.
- Immunocyto/histochemistry: 1:200-1:400. Works on live and fixed cells, tissue sections either frozen or fixed with any kind of fixative (4, 5, 6).
- Cell sorting and cell panning (8)
- RIA (7)

Optimal working dilutions must be determined by end user.

SPECIES REACTIVITY: The monoclonal works well on all species expressing PSA-NCAM including mouse.
FORMAT: Ascites in 50 % glycerol.

STORAGE: Maintain at -20°C in undiluted aliquots for up to 12 months. Avoid repeated freeze/thaw cycles.





A,B PSA-NCAM expression at the surface of neonatal brain precursors (red) with many BrdU nuclei (green). A Magnification 530X
B Magnification 125X [8]

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