

**raVO** PNS+2 Blot

Recombinant Immunoblot for the Detection of Autoantibodies to GAD65 and for the Detection of the Paraneoplastic Autoantibodies anti-HuD, anti-Yo, anti-Ri, anti-CV2 (anti-CRMP5), anti-Amphiphysin, anti-Ma1, anti-Ma2 and anti-SOX1

**8, 16 or 24 Determinations**

**Version 10/2013**

**Please pay attention to the differences in comparison to the previous version 02/2012**

Page 2: Reagents: more detailed description

Page 6: Procedure: More detailed description of wash steps.

## **Reagents:**

<b>Component</b>	<b>Amount/Volume</b>	<b>Description</b>
Nitrocellulose-Strips	8 (PNS+2-001) 16 (PNS+2-002) 24 (PNS+2-003)	Nitrocellulose-Strips for the detection of IgG antibodies, purple screw cap
Wash buffer	1 x 50 ml (PNS+2-001) 2 x 50 ml (PNS+2-002) 3 x 50 ml (PNS+2-003)	Wash buffer, 20 x concentrated, blue screw cap
Dilution buffer for samples	1 x 25 ml (PNS+2-001) 2 x 25 ml (PNS+2-002) 3 x 25 ml (PNS+2-003)	ready to use, contains 0,03% ProClin300, green screw cap
Positive control	1 x 2 ml (PNS+2-001) 2 x 2 ml (PNS+2-002) 3 x 2 ml (PNS+2-003)	ready to use, contains ProClin300, blue screw cap
Conjugate for samples and positive control	1 x 20 ml (PNS+2-001) 2 x 20 ml (PNS+2-002) 3 x 20 ml (PNS+2-003)	ready to use, Alkaline Phosphatase IgG-Conjugate contains 0,03% ProClin300, white screw cap
Substrate	1 x 20 ml (PNS+2-001) 2 x 20 ml (PNS+2-002) 3 x 20 ml (PNS+2-003)	BCIP/NBT ready to use, black screw cap
Incubation trays	1 x (PNS+2-001) 2 x (PNS+2-002) 3 x (PNS+2-003)	
Instruction for use	1 x	

## **Additional reagents available on request:**

Negative Control	2 ml	ready to use, contains 0,03% ProClin300 Colourless screw cap
Product No.:	PNSNKO	

## **Summary:**

Paraneoplastic neurological syndromes (PNS) are a group of neurological disorders associated with a tumor and its metastasis that are not the cause of the syndromes. An autoimmune process is considered as the underlying pathophysiological mechanism. Specific antineuronal autoantibodies can be detected in a majority of patients with PNS. Demonstration of the well characterized autoantibodies **anti-HuD, anti-Yo, anti-Ri, anti-CV2/CRMP5, anti-Amphiphysin, anti-Ma1 and anti-Ma2** in the presence of paraneoplastic neurological symptoms provides strong diagnostic evidence of a – possibly occult – neoplasm. In two-thirds of the cases, a paraneoplastic neurological syndrome precedes the discovery of an underlying neoplasm by up to five years. Thus, demonstration of paraneoplastic antineuronal autoantibodies may lead to an early discovery of cancer (1-11, table).

**SOX1:** In up to 50 % of patients with Lambert-Eaton myasthenic syndrome (LEMS) - a disorder mediated by voltage-gated calcium channels (VGCC) - cancer is detected, almost always a small cell lung cancer (SCLC). Recently it has been shown that in 43 % of patients with LEMS and SCLC the detectable antibodies (AGNA = anti-glial nuclear antibodies) are directed to SOX1. SOX1 is a protein which plays a role in neuronal development and which is expressed in SCLC (12). In single cases autoantibodies to SOX1 have been detected in patients with paraneoplastic limbic encephalitis. Antibodies to SOX1 can also be detected in other paraneoplastic neurological syndromes in association with the well characterized antineuronal autoantibodies.

**GAD65:** Antibodies to GAD (glutamat-decarboxylase) are considered as serological markers for Stiff-Person-Syndrome a rare neurological autoimmune disease that is characterized by rigidity and episodic spasms of muscles as a result of continuous motor unit activity. The majority of patients have high titers of antibodies to both isoforms, GAD65 and GAD67. These enzymes catalyse the conversation of glutamate to GABA ( $\gamma$ -aminobutyric acid), a major inhibitory neurotransmitter of the CNS.

High titer GAD antibodies have recently been described to define a form of non-paraneoplastic Limbic Encephalitis (13).

**Table:****Paraneoplastic neurological syndromes****Most frequently associated tumors**

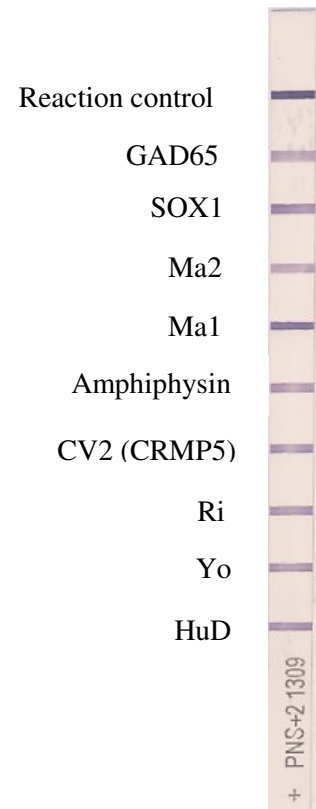
Anti-Hu-Antibodies (ANNA-1)	<ul style="list-style-type: none"> <li>• <b>Sensory and autonomic neuropathy</b></li> <li>• <b>Cerebellar ataxia</b></li> <li>• <b>Encephalomyelitis</b></li> <li>• <b>Limbic Encephalitis</b></li> </ul>	Small-cell-lung cancer Non-small-cell lung cancer Extrapulmonary small cell cancer
Anti-Yo-Antibodies (Purkinje-cell-antigen)	<ul style="list-style-type: none"> <li>• <b>Cerebellar ataxia</b></li> </ul>	Breast cancer Ovarian cancer Uterus cancer
Anti-Ri-Antibodies (ANNA-2, anti-Nova-1)	<ul style="list-style-type: none"> <li>• <b>Brainstem encephalitis (incl. Opsoclonus-Myoclonus-Syndrome)</b></li> <li>• <b>Cerebellar ataxia</b></li> </ul>	Breast cancer Small-cell-lung cancer Medullary carcinoma of the thyroid gland
Anti-CV2-(CRMP5-) Antibodies	<ul style="list-style-type: none"> <li>• <b>Sensory and sensorimotor neuropathy</b></li> <li>• <b>Encephalomyelitis</b></li> <li>• <b>Cerebellar ataxia</b></li> <li>• <b>Limbic Encephalitis</b></li> <li>• <b>Autonomic neuropathy</b></li> <li>• <b>Chorea</b></li> </ul>	Small-cell-lung cancer Thymom
Anti-Amphiphysin-Antibodies	<ul style="list-style-type: none"> <li>• <b>Stiff-person-syndrom</b></li> <li>• <b>Various symptoms</b></li> </ul>	Breast cancer Small-cell-lung cancer
Anti-Ma1 and Anti-Ma2- (Ta-) Antibodies	<ul style="list-style-type: none"> <li>• <b>Limbic Encephalitis</b></li> <li>• <b>Brainstem encephalitis*</b></li> <li>• <b>Cerebellar ataxia*</b></li> </ul>	Testicular cancer Lung-cancers
Anti-SOX1-Antibodies	<ul style="list-style-type: none"> <li>• <b>Lambert Eaton Myasthenia gravis</b></li> </ul>	Small-cell-lung cancer
Anti-GAD65-Antibodies	<ul style="list-style-type: none"> <li>• <b>Stiff-Person-Syndrom</b></li> <li>• <b>Limbic Encephalitis</b></li> </ul>	Non paraneoplastic

\* Brainstem encephalitis and cerebellar ataxia usually associated with tumors different from testicular and immunoreactivity against Ma2 and Ma1 proteins.

### **Principle:**

Nitrocellulose strips which are coated with the recombinant antigens HuD, Yo, Ri, CV2 (CRMP5), Amphiphysin, Ma1, Ma2, SOX1 and GAD65 are incubated with a specimen of patient serum.

Specific antibodies in the specimen will bind to the antigens. Non specific molecules in serum specimens will be removed by washing the strips. Bound antibodies are detected by alkaline phosphate conjugated anti-IgG using BCIP/NBT as substrate.



### **Storage:**

All kit components are stable until date of expiry stored at +4....8°C.

### **Reconstitution:**

- Make sure all kit components are at room temperature before use.
- Dilute the wash buffer concentrate 1:20 with distilled water. **During storage at low temperatures, crystals may form in concentrated wash buffers, which can be dissolved by incubating the concentrate at 37°C for 30 minutes.** Diluted wash buffer is stable for 4 weeks stored at +4 to +8°C.
- Dilute specimen **1: 2,000** in two steps in ready to use dilution buffer for samples  
1. Add 10 µl specimen to 90 µl ready to use dilution buffer, mix well. 2. See below  
**The positive control is ready to use. No further dilution is necessary.**
- The testing of cerebrospinal fluid (CSF) and the detection of intrathecal specific autoantibody synthesis is described on page 6

**Procedure:**

The Nitrocellulose strips are labeled at the bottom (if not indicated otherwise). They must be incubated with the labels facing upwards and should be completely covered with fluid during all incubation steps.

**Add the ready to use positive control (2ml) to one strip  
Cover the other strips with 2 ml dilution buffer. Add 10 µl of the pre-diluted specimen and mix carefully (end-dilution: 1 : 2,000)  
Incubate for 60 minutes at room temperature on a rocking table.**

- wash with diluted wash buffer: Carefully remove the fluid using a pipet or pour away the fluid from each strip. Add ca. 2 ml of diluted wash buffer to each strip and shake for ca. 30 seconds. Repeat five times.
- Add 2 ml alkaline phosphatase IgG conjugate, ready to use, per strip.

**Incubate for 30 minutes at room temperature on a rocking table.**

- wash with diluted wash buffer: Carefully remove the fluid using a pipet or pour away the fluid from each strip. Add ca. 2 ml of diluted wash buffer to each strip and shake for ca. 30 seconds. Repeat five times.
- Incubate each strip in 2 ml ready to use substrate-solution.

**Incubate for 25 minutes at room temperature until the bands become clearly visible.  
See control scan for comparison.**

- Transfer the strips to distilled water to stop the reaction. Put the strips onto filter paper and let them dry. Store the strips in the dark.

**Testing of cerebrospinal fluid (CSF), detection of intrathecal specific autoantibody synthesis:**

- Determination of the total IgG concentration of a serum-CSF-sample pair
- Dilute both, serum and CSF, in ready to use sample buffer to a concentration of 1 mg/liter as working solution (see example)
- Perform test in parallel as described on page 3. Use 2 ml each of the respective calculated dilution.

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Example:

1 mg/L	Determined conc.of IgG	Dilution
CSF	97,3 mg/L	1: 97
Serum	10,8 g/L	1 : 10.000

Compare the intensity of the bands of the serum-CSF-pair.

A more intensive band for the CSF-sample hints at an intrathecal specific antibody synthesis. If the intensity of the serum-CSF-pair is almost equal, run of the test at an IgG-concentration of 0.1 mg/liter may be helpful for easier interpretation.

### **Interpretation:**

Put the strips side by side to assign the proteins. Only strips from the same batch should be compared.

The positive control and control scan helps to assign the proteins and to stick the strips on the latter for documentation.

Usually strong bands are observed in serum samples from patients with clinically defined paraneoplastic symptoms.

The meaning of weak reactions is still unknown with the exception of autoantibodies to HuD. For HuD- auto-antibodies weak reactions are described in 18% of patients suffering from small cell lung cancer without paraneoplastic neurological disease. Thus, in case of low concentrations of anti-Hu antibodies a thorough search for a possible underlying tumor is recommended. Concerning weak reactions with the other antigens there is nothing known at present about the incidence of underlying tumours so that no clear recommendation can be given.

At least a control of the antibody status is recommended in the course of the disease.

### **Ma1/Ma2 Reactivity**

#### **Ma1 - / Ma2 +:**

If patient sera are reactive with the Ma2 antigen exclusively this hints at a testicular cancer as underlying malignancy.

#### **Ma1 + /Ma2 + :**

The presence of both antibodies specific for Ma1 and Ma2 antigens proofs a paraneoplastic etiology of a neurological disease but it does not hint at a special type of cancer.

In contrast to the well-known paraneoplastic autoantibodies low antibodies titers to SOX1 are sometimes detected. The meaning of these weak reactions is unknown at the moment.

Antibodies to GAD (glutamat-decarboxylase) are considered as serological markers for Stiff-Person-Syndrome. The majority of patients have high titers of antibodies to both isoforms, GAD65 and GAD67.

High titer GAD antibodies have recently been described to define a form of non-paraneoplastic Limbic Encephalitis.

### **Remarks:**

- Avoid contact of the skin with substrate solution.

## References:

1. O,Stich, S.Rauer. Paraneoplastische Neurologische Syndrome. Der Nervenarzt. 2013; 4
2. S. Rauer. Paraneoplastische Neurologische Syndrome. Fortschr Neurol Psychiatr. 2011;79(1):51-8; quiz 59-61. Review.
3. Oliver Stich, Sven Jarius, Christiane Rasiah, Raymond Volz und Sebastian Rauer. Recombinant immunoblot for assessment of intrathecally synthesized paraneoplastic antineural antibodies in cerebrospinal fluid from patients with paraneoplastic neurological syndromes. Clin Chem Lab Med 2008;46(12):1793-1795.
4. Lisa M. DeAngelis and Jerome B. Posner. Neurologic Complications of Cancer. Oxford University Press 2008 Second Edition
5. F. Graus, J.Y. Delattre, J. C. Antoine, J. Dalmau, B. Giometto, W. Grishold, J. Honnorat, P. Sillevs Smitt, Ch. Vedeler, J. J. G. M. Verschuuren, A. Vincent, R. Voltz, for the Paraneoplastic Neurological Syndrome Euronetwork. Recommended diagnostic criteria for paraneoplastic neurological syndromes. J Neurol Neurosurg Psychiatry 2004;75:1135-1140.
6. Voltz R. Paraneoplastische neurologische Autoimmunerkrankungen. Nervenarzt 2002;73:909 – 929.
7. Honnorat J., Antoine J.C., Derrington E., Aguqra M., Belin M.F. Antibodies to a subpopulation of glial cells and a 66 kDa developmental protein in patients with paraneoplastic neurological syndromes. J.Neurol.Neurosurg.Psychiatry 1996;61(3): 270-8.
8. Sillevs-Smitt P., Manley G., Dalmau J., Posner J.B.Pitfalls in the diagnosis of autoantibodies associated with paraneoplastic neurologic disease. Neurology 1996;46:1739-1741.
9. Moll J.W., Antoine J.C., Brashear H.R. et al. Guidelines on the detection of paraneoplastic anti-neuronal-specific antibodies: report from the Workshop to the fourth Meeting of the International Society of Neuro-Immunology on paraneoplastic neurological disease. Neurology 1995;45:1937-1941.
10. Moll J.W., Vecht C.J. Immune diagnosis of paraneoplastic neurological diseases. Clin. Neurol. Neurosurg.1995;97: 711-81.
11. Dalmau J., Posner J.B. Neurologic paraneoplastic antibodies (anti-Yo; anti-Hu; anti-Ri): the case for a nomenclature based on antibody and antigen specificity.Neurology 1994;44:2241-2246.
12. L. Sabater, M.Titulaer, A, Saiz, J.Verschuuren, A. O. Güre and F. Graus. SOX1 antibodies are markers of paraneoplastic Lambert-Eaton myasthenic syndrome. Neurology, 2008; 70: 924-928.
13. Michael P. Malter, Christoph Helmstaedter, Horst Urbach, Angela Vincent, and Christian G.Bien. Antibodies to Glutamic Decarboxylase Define a Form of Limbic Encephalitis. Ann Neurol 2010;67:470-478.

Product-No: PNS+2-001 ( 8 Determinations)  
PNS+2-002 (16 Determinations)  
PNS+2-003 (24 Determinations)

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