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. 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.
The presence of anti-Amphiphysin antibodies (with or without GAD) indicates a paraneoplastic neurological syndrome caused by an underlying tumour.

Remarks:

- Avoid contact of the skin with substrate solution.

Product-No: SPB001 (8 Determinations)
SPB002 (16 Determinations)
SPB003 (24 Determinations)

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08/2012

ravo Diagnostika GmbH
CE

ravo Stiff Person Blot
Recombinant Immunoblot for the Detection of anti-Amphiphysin and anti-GAD65 autoantibodies for the diagnosis of stiff person syndrome

8, 16 or 24 Determinations

Version 08/2012
Please pay attention to the differences in comparison to the previous version 03/2012

Page 1: Reagents
1. No more conjugate for the positive control required.
2. White screw cap for the Alkaline Phosphatase conjugated anti-human-IgG

Page 2: Procedure
1. No more conjugate for the positive control required.

Summary:

Stiff-Person Syndrome is a rare neurological autoimmune disease that is characterized by rigidity and episodic spasms of muscles as a result of continuous motor unit activity.
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. These enzymes catalyse the conversation of glutamate to GABA (γ-aminobutyric acid), a major inhibitory neurotransmitter of the CNS.
Amphiphysin expression is found in synaptic vesicels of neurons as well as in the sceletal musculature.
The presence of anti-Amphiphysin antibodies (with or without GAD) indicates a paraneoplastic neurological syndrome caused by an underlying tumour.

Principle:

Nitrocellulose strips which are coated with the recombinant antigens Amphiphysin 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-human IgG using BCIP/NBT as substrate.

Reagents:

- Nitrocellulose-Strips for the detection of IgG-antibodies
- 1 control-scan
- Wash buffer concentrate, blue screw cap
- Dilution Buffer for samples, ready to use, contains 0.01% ProClin300, green screw cap
- Positive Control, ready to use, contains 0.01% ProClin300, purple screw cap
- Negative control, ready to use, contains 0.01% ProClin300, colourless screw cap
- Alkaline Phosphatase conjugated anti-human-IgG, ready to use, contains 0.01% ProClin300, white screw cap
- Substrate Solution (BCIP/NBT), ready to use, black screw cap
- Incubation tub(s)
- Instructions for use

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.

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.

Cover one strip each with the ready to use positive and negative control (2 ml)
Cover the other strips with 1.5 ml dilution buffer. Add 15 µl of specimen and mix carefully (end-dilution: 1 : 100)
Incubate for 60 minutes at room temperature on a rocking table.

- wash five times with diluted wash buffer.

- Add 2 ml alkaline phosphatase IgG conjugate, ready to use, per strip.

Incubate for 30 minutes at room temperature on a rocking table

- wash five times with diluted wash buffer.

- Incubate each strip in 2 ml ready to use substrate-solution.

Incubate for 25 minutes at room temperature.

- 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.