Diagnostika GmbH

CE

ravo PKCγ Line Assay
Recombinant Line Assay for the Detection of Autoantibodies to the Protein Kinase C gamma

8, 16 or 24 Determinations

Product-No: PKC001 (8 Determinations)
PKC002 (16 Determinations)
PKC003 (24 Determinations)

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Summary:

Autoantibodies to Protein Kinase C gamma (PKCγ), an enzyme of ~ 80 kD have been detected in patients with paraneoplastic cerebellar degeneration. The immunefluorescence staining pattern shows staining of the cytoplasma, dendrites and axons of Purkinje-cells[1,2].

Principle:

Nitrocellulose strips which are coated with highly purified recombinant PKCγ are incubated with a specimen of patient serum. Specific antibodies in the specimen will bind to the antigen. 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:

Component	Amount/Volume	Description
Nitrocellulose-Strips	8 (PKC001) 16 (PKC002) 24 (PKC003)	Nitrocellulose-Strips for the detection of IgG antibodies, black screw cap
Wash buffer	1 x 50 ml (PKC001) 2 x 50 ml (PKC002) 3 x 50 ml (PKC003)	Wash buffer, 20 x concentrated, blue screw cap
Dilution buffer for samples	1 x 25 ml (PKC001) 1 x 25 ml (PKC002) 2 x 25 ml (PKC003)	ready to use, contains 0,03% ProClin300, green screw cap
Conjugate for samples and positive control	1 x 20 ml (PKC001) 2 x 20 ml (PKC002) 3 x 20 ml (PKC003)	ready to use, Alkaline Phosphatase Conjugate contains 0,03% ProClin300, red screw cap
Substrate	1 x 20 ml (PKC001) 2 x 20 ml (PKC002) 3 x 20 ml (PKC003)	BCIP/NBT ready to use, black screw cap
Incubation trays	1 x (PKC001) 2 x (PKC002) 3 x (PKC003)	
Instruction for use	1 x	

Additional reagents available on request:

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

Storage:

All kit components and unopened opened are stable until date of expiry stored at +2....8°C.
Diluted wash buffer is stable for 4 weeks stored at +2 to +8°C.

Specimen:

Preferably freshly collected specimen (serum or plasma) should be used. Specimen may be stored at 2 – 8°C for up to 5 days. For longer storage at 2 – 8°C antimicrobial agents (e.g. Thimerosal at a final dilution of 0.01%, ProClin300 at a final dilution of 0.03% or NaN_3 at a final dilution of 0.09%) should be added.
For long term storage (several months) specimen should be stored in aliquots at – 20°C. Avoid repeated freeze thawing.
Contaminated specimen may lead to false positive or negative results and should not be used.
Some serum samples show a more or less strong violet background due to serum components of unknown origin. Such samples should be retested using another method e.g. IFA.

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.** Let the solution cool down again to room temperature before use. Diluted wash buffer is stable for 4 weeks stored at +2 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 the other strips with 2 ml dilution buffer. Add 10 µl of the specimen and mix carefully (end-dilution: 1 : 200)
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.

- 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 20 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.

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, the documentation sheet to stick the strips on the latter for documentation.

Remarks:

- Avoid contact of the skin with substrate solution.

References:

1. R.Höftberger, G.G.Kovacs, L.Sabater, P.Nagy, G.Racz, R.Miquel, J.Dalmau and F.Graus. Protein kinase Cγ antibodies and paraneoplastic cerebellar degeneration. J Neuroimmunol, 2013;256:91-93
2. S.Jarius and B.Wildemann. 'Medusa head ataxia': the expanding spectrum of Purkinje cell antibodies in autoimmune cerebellar ataxia. Part 2: Anti-PKC-gamma, anti-GluR-delta2, anti-Ca/ARHGAP26 and anti VGCC. Journal of Neuroinflammation, 2015;12:167