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Instruction For Use
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ORG 760 Myositis plus

NAME AND INTENDED USE

Myositis plus Immunoblot assay is a membrane based enzyme immunoassay for the semi-quantitative measurement of IgG class autoantibodies to AMA-M2, Jo-1, PM-Scl-100, PL 7, PL 12, Mi-2, Ku (p70/80) and SRP in human serum or plasma. The assay is intended for professional in vitro diagnostic use only.

SYMBOLS USED

IVD	In vitro diagnostic medical device		
	Manufacturer		
REF	Catalogue number	BLOT STRIPS	Blot strips
Σ	Sufficient for	DILUENT	Sample Buffer
LOT	Batch code	CONJUGATE	Enzyme Conjugate
	Use by	WASH	Wash Buffer
2°C 8°C	Temperature limitation	BCIP	BCIP Substrate
	Consult instructions for use	RTU	Ready to use
	Keep away from sunlight		
	Do not reuse		
	Date of manufacture		

SUMMARY AND EXPLANATION OF THE TEST

Dermatomyositis, polymyositis and inclusion body myositis are idiopathic inflammatory diseases of unknown aetiology. They are characterised by muscle weakness and atrophy of the proximal musculature; progression of the disease is generally subacute. These myopathies are autoimmune diseases.

Idiopathic myositides are often associated with other autoimmune diseases and connective tissue disorders or have similar symptoms to these, which occasionally makes a clear diagnosis difficult (overlap syndrome). In up to 90 % of patients, autoantibodies are found, 60 % of which are directed against specific nuclear (ANA) and/or cytoplasmic antigens. In one fifth of cases, ribonucleoproteins involved in protein biosynthesis are the target antigens (tRNA-synthetases, SRP). These autoantibodies indicate interstitial lung disease (ILD).

Myositis-specific autoantibodies, MSA, generally only appear in cases of idiopathic inflammatory myositides and are thus markers of the disease. They may be detectable before clinical manifestation of the disease and disappear after successful treatment. Myositis-associated autoantibodies, MAA, are also found in patients suffering from diseases other than idiopathic inflammatory myositides.

Myositis plus by ORGENTEC Diagnostika features the typical and most common antigens for the detection of MSA and MAA. Through the combination of selected antigens this immunoblot test allows for the simple and rapid diagnosis of idiopathic inflammatory myositides and the differential diagnosis of symptomatically coincident and similar diseases. The antigen configuration of Myositis plus also allows for the differentiation of autoantibodies against ribosomes and mitochondria that show a cytoplasmic pattern by immunofluorescence.

AMA-M2

Anti-mitochondrial antibodies (AMA) are a heterogeneous group of autoantibodies directed against various proteins of the outer and inner mitochondrial membrane. AMA of the M2 subtype are directed against epitopes of the pyruvate dehydrogenase complex. The high sensitivity and specificity of M2 autoantibodies makes them excellent for the detection of primary biliary cirrhosis (PBC).

Anti-Jo-1

Antibodies against the Jo-1 antigen, histidyl-tRNA synthetase, are the most common myositis-specific autoantibodies (MSA). Nearly 90 % of patients in whom anti-Jo-1 is detected develop myositis in the course of their disease. Jo-1 is a cytoplasmic enzyme that catalyses the binding of histidine to the specific tRNA in protein biosynthesis.

Anti-PM-Scl-100

The myositis-associated antibodies to PM-Scl-100 are an important indicator of myositis/scleroderma overlap syndrome. They can be detected in nearly one fourth of patients with myositis/scleroderma overlap, but in cases of mere scleroderma or myositis they are rare, at 2 and 6 %, respectively. Anti-PM-Scl-100 are directed against epitopes of the exosomes, multienzyme complexes that consist of multiple RNA-binding proteins and enzymes with exoribonuclease function and that play an important role in RNA processing.

Anti-PL-7

PL-7 autoantibodies are directed against the threonyl-tRNA synthetase of the cytoplasm. Anti-PL-7 are myositis-specific antibodies, but are rare, with a prevalence of less than 3 %.

Anti-PL-12

Anti-PL-12 is another myositis-specific antibody directed against the alanyl-tRNA synthetase in the cytoplasm. Its prevalence is low at fewer than 3 %.

Anti-Mi-2

Myositis-specific antibodies against the Mi-2 protein are diagnostic markers for idiopathic myositides. About 95 % of anti-Mi-2-positive patients have dermatomyositis and 3 % have polymyositis. The target antigen of this autoantibody is the central component of the multi-protein complex NuRD (nucleosome remodelling and histone deacetylase) that catalyses the modification of the chromatin structure and thus acts as a transcription activator or repressor.

Anti-Ku (p70/80)

The target antigen for these myositis-associated antibodies is the Ku antigen, an ATP-dependent DNA helicase. Antibodies against Ku are detectable in up to 7 % of patients with myositides. They are also found in other autoimmune diseases, such as SLE or Sjögren´s syndrome.

Anti-SRP

The myositis-specific anti-SRP antibodies can be detected in cases of severe myositides. Their prevalence in myositis is about 4 %; about one fourth of anti-SRP-positive patients show the clinical signs of dermatomyositis. The target antigen is the signal recognition particle (SRP), a cytoplasmic ribonucleoprotein complex responsible for the translocation of newly synthesised proteins from the ribosomes to the endoplasmic reticulum.

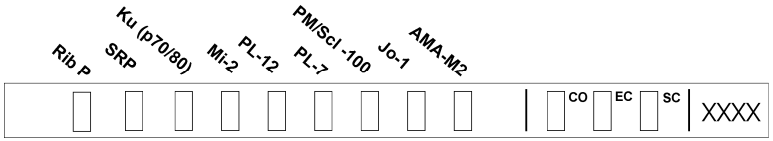
Anti-Rib-P

Rib-P antibodies are diagnostic markers of systemic lupus erythematosus. They are detectable in 10 to 20 % of SLE patients, primarily in active phases of the disease; they are rarely found in patients with other autoimmune diseases. In patients with scleroderma, the Rib-P antibodies may indicate a scleroderma-SLE overlap. The target antigens of this autoantibody are specific regions of the phosphoproteins P0 (38 kDa), P1 (19 kDa) and P2 (17 kDa) of the 60S subunit of the ribosomal complex.

PRINCIPLE OF THE TEST

Highly purified antigens AMA-M2, Jo-1, PM-Scl-100, PL 7, PL 12, Mi-2, Ku (p70/80) and SRPas well as three control antigens for CO Cut-off Control, EC Enzyme Conjugate Control and SC Serum Control are bound to nitrocellulose membrane blot strips.

Autoantibodies present in serum or plasma bind to the immoblizied antigen. Washing of the blot strips removes unbound antibodies and unspecific sample components. Alkaline phosphatase conjugated anti-human IgG detect the bound sample antibodies forming a conjugate/antibody/antigen complex. Washing of the blot strips removes unbound conjugate. The substrate BCIP/NBT is hydrolized by bound enzyme conjugate to form an insoluble blue-violet product. Washing of the blot strips removes unhydrolyzed substrate and stopps the reaction. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.



WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate BCIP/NBT.
- Sample buffer and wash buffer contain sodium azid 0.09% as preservative. This concentration is classified non-hazardous.
- Enzyme conjugate contains 0.05% ProClin as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store subtrate solution in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum by centrifugation.
- Test serum should be clear and non-hemolysed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
- Avoid repetitive freezing and thawing of serum samples.
- Testing of heat-inactivated sera is not recommended.

CONTENTS OF THE KIT

▽16	ORG 760-16	Sufficient for 16 determinations
▽8	ORG 760-08	Sufficient for 8 determinations
BLOT STRIPS	1x/2x 8	8 antigen coated nitrocellulose strips. Ready to use. 1 pre-developed calibration strip (coded CAL) for semiquantitative evaluation. Ready to use. Product code on strip: ***760***
DILUENT	1x 20 ml	Sample Buffer PB, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow. Ready to use.
CONJUGATE	1x 20 ml	Enzyme Conjugate containing anti-human IgG antibodies, alkaline phosphatase labelled; PBS, BSA, detergent, preservative ProClin 0.05%, light red. Ready to use.
WASH	1x 20 ml	Wash Buffer WB, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
BCIP	1x/2x 10 ml	BCIP Substrate; containing BCIP/NBT. Ready to use.
		Incubation tray
	1x	Instruction for Use: ELISA Mini-CD0
	1x	Certificate of Analysis

MATERIALS REQUIRED

- Pipettes for 10 µl and 1000 µl
- Distilled or deionised water
- Graduated cylinder for 1000 ml
- Laboratory timing device
- Rocking platform
- Tweezers

STORAGE AND STABILITY

- Store the kit at 2-8 °C.
- Keep nitrocellulose strips carefully sealed in the original plastic tube with desiccants provided.
- Important: The calibration strip is very light-sensitive. Store in the dark!
- Do not expose test reagents to heat, sun or strong light during storage and usage.
- The unopened test kit is stable for 18 months from day of production. See expiry date on outer labels for individual batches.
- Diluted wash buffer is stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots.
- All materials must be at room temperature (20-28 °C).
- Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions
- To avoid carryover contamination, change the tip between samples.
- All incubation steps must be accurately timed.
- Control sera should routinely be assayed as unknowns to check performance of the reagents and the assay.
- Nitrocellulose strips must be handled with gloves or tweezers.
- It is important to make sure, that air-bubbles do not interfere with the strip during incubation. This could cause irregularities in coloration of developing bands and can lead to wrong results.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Ready to use.

Preparation of samples

Sample dilution see test procedure. Effective dilution during test is 1:101.

TEST PROCEDURE

Using tweezers insert one nitrocellulose strip into one chamber of the incubation tray:

- Add **1.0 ml sample buffer** to the strip in the chamber.
- Allow to equilibrate for 5 minutes with gentle bobbing.
- Add **10 µl of patient sample** directly to the chamber.
- Incubate for **60 minutes** at room temperature (20-28 °C) with gentle bobbing.
- Remove the diluted sample completely from the chamber.
- Add 2.0 ml wash buffer to the chamber, incubate for 5 minutes.
- Remove wash buffer completely. Repeat this procedure twice.

- Add **1.0 ml enzyme conjugate** to each strip in the chamber of the incubation tray.
- Incubate for **30 minutes** at room temperature with gentle bobbing.
- Remove the conjugate completely from the chamber.
- Add 2.0 ml wash buffer to the chamber, incubate for 5 minutes.
- Remove wash buffer completely. Repeat this procedure twice.

- Add **1.0 ml substrate** to each strip in the chamber of the incubation tray.
- Incubate for **10 minutes** at room temperature with gentle bobbing.
- Remove the substrate completly.
- Add 1.0 ml distilled water to the chamber, incubate for 5 minutes.
- Remove water completely. Repeat this procedure twice.

Carefully blot the strips with a tissue paper. Allow strips to air dry before evaluating with the calibration strip.

VALIDATION

The assay is valid if the all three control lines (**CO** Cut-off Control, **EC** Enzyme Conjugate Control and **SC** Serum Control) show a turn-over of substrate in terms of blue-violet lines! If this criteria is not met the assay is invalid and should be repeated.

Note: Borderline samples should be repeated or tested using an alternative procedure. Samples from patients diagnosed with autoimmune diseases often show multiple autoantibody specificities. Such samples may show a positive reaction with more than one antigen line.

CALCULATION OF RESULTS

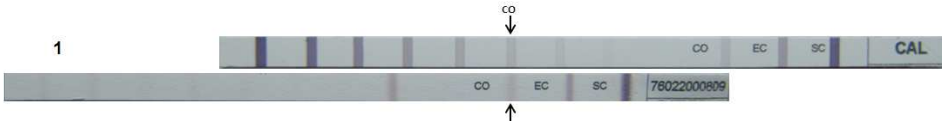
The intensity of a **blue-violet line** at the position of the coated antigen is directly proportional to the concentration of IgG antibodies present in the sample tested.

Semi-quantitative evaluation of sample strip:

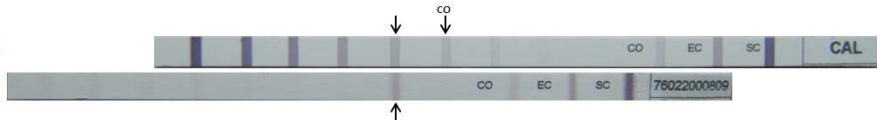
negativ	intensity of patient sample line weaker than intensity of CO-line
borderline	intensity of patient sample line eqivalent to intensity of CO-line
weak positive	intensity of patient sample line up to 1 level stronger than intensity of CO-line
positive	intensity of patient sample line up to 2 levels stronger than intensity of CO-line
strong positive	intensity of patient sample line ≥3 levels stronger than intensity of CO-line

Interpretation of the intensity of blue-violet lines:

(1) Compare intensity of the **CO-line of the sample strip** to the intensity of the lines of the calibration strip.
Example:



(2) Compare the intensity of the **patient sample line** to the intensity of the lines of the calibration strip.
Example: Interpretation of intensity of patient sample line is "weak positive".



PERFORMANCE CHARACTERISTICS

CALIBRATION

The sensitivity, specificity and dose response of the Myositis plus immunoblot was evaluated using clinically defined in house quality control sera containing varying relative amounts of sera with known specificity.

Measuring range

The evaluation of the intensity of the blue lines as described above allows a semi-quantitative determination of IgG class autoantibodies in the sample tested into quantification ranges:

negative, borderline, weak positive, positive, strong positive

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this assay. Cut-off: borderline

Interpretation of results

normal:	negative
elevated:	weak positive, positive, strong positive

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity of each dilution step was determined using the calibration strip.

Linearity				
Sample	Dilution	Observed	Expected	O/E
1	1:100	strong positive	strong positive	PASS
.	1:200	positive	positive	PASS
.	1:400	weak positive	weak positive	PASS
.	1:800	borderline	borderline	PASS
.	1:1600	negative	negative	PASS
2	1:100	strong positive	strong positive	PASS
.	1:200	positive	positive	PASS
.	1:400	weak positive	weak positive	PASS
.	1:800	borderline	borderline	PASS
.	1:1600	negative	negative	PASS

Sensitivity

This immunoblot assay is a semi-quantitative assay method. Any reactivity less than borderline is considered

negative and cannot be quantified any further.

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean	Result
1	negative	PASS
2	weak	PASS
3	positive	PASS

Inter-Assay		
Sample	Mean	Result
1	negative	PASS
2	weak	PASS
3	positive	PASS

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n pos	%
Poly-or dermatomyositis	41	28	68.3
Primary biliary cirrhosis (PBC)	17	13	76.5
Rheumatoid Arthritis (RA)	51	0	0.0
Normal human sera	64	7	10.9

			Clinical Diagnosis Pos	Clinical Diagnosis Neg	
ORG 760	Pos		41	7	
Myositis plus	Neg		17	108	
.	.		58	115	173
Sensitivity:	70.7	%			
Specificity:	93.9	%			
Overall agreement:	86.1	%			

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

REFERENCES

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1. Add **blot strip** into the incubation tray
   → Add **1000 µl** sample buffer per strip into the incubation tray
   → Shake **5 minutes** while incubating

2. Add **10 µl** patient sample and resuspend
   → Shake **60 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **2000 µl** wash buffer, discard wash

3. Add **1000 µl** enzyme conjugate solution per strip
   → Shake **30 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **2000 µl** wash buffer, discard wash

4. Add **1000 µl** substrate per strip
   → Shake **10 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **1000 µl distilled water**, dry blot strips. Read after complete drying, only