A-Cube®
Academic material

FUSHIMI Pharmaceutical Co.,Ltd.
Clinical Laboratory Center

ProteoBridge Co., Ltd.

2022.12 Revised

Instructions for Research and Examination

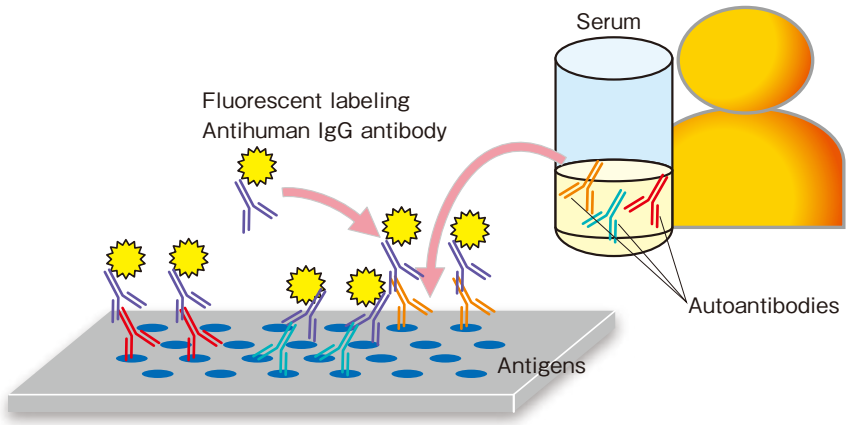
Examination Items	Detection antibody (antigen)
A-Cube Systemic Sclerosis Related Antibodies Detection Kit (SSc) 33 Antigens	CENP-A (CENPA), CENP-B (CENPB), CENP-C (CENPC), Scl-70/TopoI (TOP1), RNAPⅢ/RPC155 (POLR3A), RNAPⅢ/RPC62 (POLR3C), RNAPI (POLR1A), RNAPⅡ (POLR2A), Th/To/7-2RNP (POP1, RPP25), U3-RNP/Fibrillarin (FBL), hUBF/NOR90 (UBTF), U11/U12-RNP (RNPC3), SSSCA1 (SSSCA1), eIF2B (EIF2B2), AMA-M2/Mitochondria M2 (DLAT, DLST, DBT, PDHX), p80-coilin (COIL) *U1-RNP_70 (SNRNP70), U1-RNP_A (SNRPA), U1-RNP_C (SNRPC), U2-RNP (SNRPB2), Ku (XRCC5, XRCC6), PM-Scl100 (EXOSC10), PM-Scl75 (EXOSC9), RuvBL1/2 (RUVBL1&2), Ki (PSME3), SS-A/Ro52 (TRIM21), SS-A/Ro60 (TROVE2), SS-B (SSB)*
A-Cube Dermatomyositis/ Polymyositis Related Antibodies Detection Kit (DM/PM) 47 Antigens	Jo-1 (HARS), PL-7 (TARS), PL-12 (AARS), EJ (GARS), KS (NARS), OJ (IARS, EPRS, LARS, MARS, QARS, KARS, RARS, DARS, AIMP1, 2, 3), Zo (FARSA, FARSB), Ha (YARS), SRP (SRP54, 14, 19, 68, 72), Mi-2 (CHD3, CHD4), TIF1-γ/p155 (TRIM33), TIF1-α/p140 (TRIM24), TIF1-β (TRIM28), MJ/NXP-2 (MORC3), SAE (SAE1, UBA2), SMN (SMN1), cN1A (NT5C1A) *U1-RNP_70 (SNRNP70), U1-RNP_A (SNRPA), U1-RNP_C (SNRPC), U2-RNP (SNRPB2), Ku (XRCC5, XRCC6), PM-Scl100 (EXOSC10), PM-Scl75 (EXOSC9), RuvBL1/2 (RUVBL1&2), Ki (PSME3), SS-A/Ro52 (TRIM21), SS-A/Ro60 (TROVE2), SS-B (SSB)*
A-Cube Multiplex Test Kit (DM/PM + SSc Related Antibodies) 67 Antigens	SSc-only 20 antigens + DM/PM only 34 antigens + Common 13 antigens total 67 antigens

※Italic:detectable antibodies (antigens) shared between SSc and DM/PM

This is an indirect fluorescent antibody test that uses nondried antigenic proteins and can detect multiple autoantibodies individually and comprehensively at a time from small amounts of serum to conventional products. This test allows detection of autoantibodies associated with systemic scleroderma and dermatomyositis/polymyositis, and the results of the test report the quantitative value (index value) of antibodies to each antigen.

※This test chip was developed and named A-Cube (Autoantibody Array Assay) under the supervision of Dr. Yoshizaki and Professor Sato of Dermatology, Graduate School of Medicine, University of Tokyo.

※This test technology was developed by the National Institute of Industrial Technology, and the test results will be provided by ProteoBridge Co., Ltd., an AIST venture.



List of detected antibodies and antigens

Detection antibody	Antigen
CENP-A	CENPA
CENP-B	CENPB
CENP-C	CENPC
Scl-70(TopoⅠ)	TOP1
RNAPⅢ(RPC155)	POLR3A
RNAPⅢ(RPC62)	POLR3C
RNAPⅠ	POLR1A
RNAPⅡ	POLR2A
Th/To(7-2RNP)	POP1
	RPP25
U3-RNP(Fibrillarin)	FBL
hUBF(NOR90)	UBTF
U11/U12-RNP	RNPC3
SSSCA1	SSSCA1
eIF2B	EIF2B2
AMA-M2(Mitochondria M2)	DLAT
	DLST
	DBT
	PDHX
p80-coilin	COIL
U1-RNP_70	SNRNP70
U1-RNP_A	SNRPA
U1-RNP_C	SNRPC
U2-RNP	SNRPB2
Ku	XRCC5
	XRCC6
PM-Scl100	EXOSC10
PM-Scl75	EXOSC9
RuvBL1/2	RUVBL1&2
Ki	PSME3
SS-A/Ro52	TRIM21
SS-A/Ro60	TROVE2
SS-B	SSB

Detection antibody	Antigen
Jo-1	HARS
PL-7	TARS
PL-12	AARS
EJ	GARS
KS	NARS
OJ	IARS
	EPRS
	LARS
	MARS
	QARS
	KARS
	RARS
	DARS
	AIMP1
	AIMP2
	AIMP3
Zo	FARSA
	FARSB
Ha	YARS
SRP	SRP54
	SRP14
	SRP19
	SRP68
	SRP72
Mi-2	CHD3
	CHD4
TIF1-γ(p155)	TRIM33
TIF1-α(p140)	TRIM24
TIF1-β	TRIM28
MJ(NXP-2)	MORC3
SAE	SAE1
	UBA2
SMN	SMN1
cN1A	NT5C1A

□ + □ Systemic Sclerosis Related Antibodies Detection Kit (SSc) 33 Antigens

□ + □ Dermatomyositis/Polymyositis Related Antibodies Detection Kit (DM/PM) 47 Antigens

□ + □ + □ Multiplex Test Kit (DM/PM + SSc Related Antibodies) 67 Antigens

Systemic Sclerosis Related Antibodies Detection Kit (SSc)

More than 90% of patients with systemic scleroderma are positive for antinuclear antibodies. Among these antinuclear antibodies, anti-centromere antibodies, anti-topoisomerase I antibodies, and anti-RNA polymerase III antibodies are covered as specific and representative of systemic scleroderma and are routinely used in scleroderma diagnosis and treatment decision making.
In this kit, systemic scleroderma-specific antibodies can be detected in RNAPI, RNAPII, Th/To, U3-RNP (Fibrillarin), hUBF (NOR90), U11/U12-RNP, and eIF2B that cannot be measured by insurance-listed tests other than those listed above. These autoantibodies have also been reported to be closely related to the clinical presentation.
Systemic scleroderma-related antibodies include anti-U1RNP antibodies (70, A, and C), anti-U2RNP antibodies, anti-Ku antibodies, anti-PM-Scl antibodies (100, 75), anti-RuvBL1/2 antibodies, and anti-Ki antibodies detected by overlap with myositis.
They can also detect anti-SSSCA1 (P27) antibodies, which are detected in scleroderma and Sjögren's syndrome, and anti-AMA-M2 (mitochondrial) antibodies (four antigens) and anti-p80-coilin antibodies, which are detected in primary biliary cirrhosis, which is highly complicated with scleroderma.
As such, this test provides important information for typing, selecting treatment, and predicting prognosis because specific and related autoantibodies for systemic scleroderma can be comprehensively measured at one time.

Performance correlation data

Percent agreement with samples determined by immunoprecipitation (mean of three autoantibodies each).

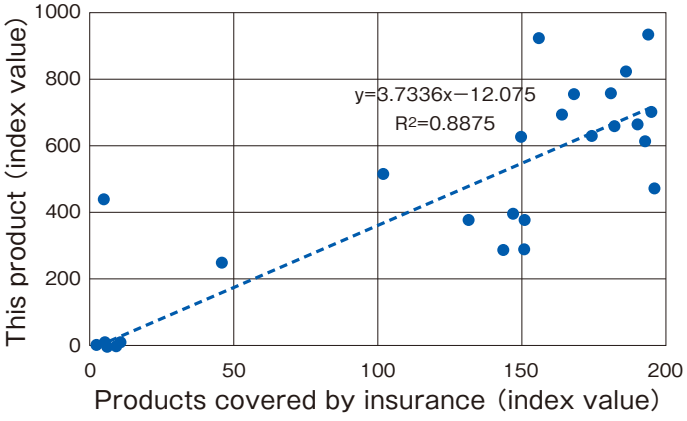
- Anti-Th/To Ab
- Anti-U3-RNP Ab
- Anti-NOR90 Ab
- Anti-PM-Scl Ab
- Anti-topoisomerase I (Scl-70) Ab
- Anti-RNA-polymerase I/III/II Ab
- Anti-Ku Ab
- Anti-SS-A Ab
- Anti-SS-B Ab

Concordance rate_100%(25/25)

Concordance rate with insurance-listed kits

▶Anti-CENP-B Ab

96 samples
Correlation coefficient r=0.942

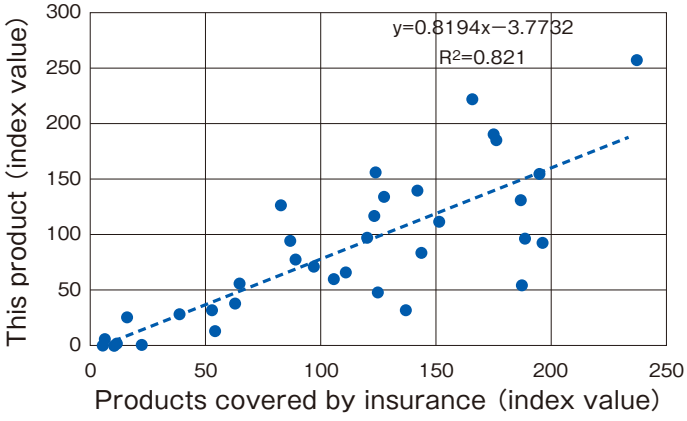


		ELISA (insured products)		
		+	±	−
A-Cube (This Product)	+	20	0	1
	±	0	1	1
	−	0	0	73

Concordance rate_98%

▶Anti-Scl-70 Ab

98 samples
Correlation coefficient r=0.906



		ELISA (insured products)		
		+	±	−
A-Cube (This Product)	+	29	1	0
	±	0	0	0
	−	1	1	67

Concordance rate_98%

Reference

Systemic Sclerosis Related Antibodies Detection Kit (SSc)

Antibody	Contents of the report	Reference
CENP-A	30% positive rate in scleroderma, localized scleroderma, skin ulcer, calcification, reflux esophagitis, pulmonary hypertension correlated with good prognosis	Japanese Journal of Clinical Immunology, 36(3)139-147(2013)
CENP-B		
CENP-C		
Scl-70(TopoI)	40% positive rate in scleroderma, diffuse type of skin sclerosis,interstitial pneumonia (pulmonary fibrosis), correlated with renal crisis,poor prognosis	Japanese Journal of Clinical Immunology, 36(3)139-147(2013)
RNAPⅢ(RPC155)	20% positive rate in scleroderma, diffuse type of skin sclerosis, correlated with renal crisis, good prognosis	Japanese Journal of Clinical Immunology, 36(3)139-147(2013)
RNAPⅢ(RPC62)		
RNAPⅠ		
RNAPⅡ		
Th/To(7-2RNP)	2-5% positivity in scleroderma, localized scleroderma, interstitial pneumonia (pulmonary fibrosis), and pulmonary arterial hypertension	Japanese Journal of Clinical Immunology, 36(3)139-147(2013)
U3-RNP (Fibrillarin)	4-10% positive rate in scleroderma, diffuse type of skin sclerosis and peripheral circulatory disturbances, interstitial pneumonia (pulmonary fibrosis), pulmonary arterial hypertension, correlate with lower gastrointestinal lesions	Japanese Journal of Clinical Immunology, 36(3)139-147(2013)
hUBF(NOR90)	10% positive rate in rheumatic diseases, 3.3% of them have scleroderma, localized skin sclerosis, correlate with non-serious visceral lesions	Arthritis Rheum.1996 Aug;39(8):1313-8. Japanese Journal of Clinical Immunology, 36(3)139-147(2013)
U11/U12-RNP	3.2% positivity in scleroderma, correlated with interstitial pneumonia (pulmonary fibrosis)	Arthritis Rheum. 2009 July 15;61(7):958-965.
SSSCA1	1.7% positivity for all autoimmune diseases, all positive for scleroderma or Sjögren's syndrome, and a subset of anti-CENP antibodies	Clin Exp Immunol. 1998 Feb;111(2):372-6.
eIF2B	A positive rate of 1-2% in scleroderma,diffuse scleroderma and interstitial lung disease were frequent,overlapping myositis or rheumatoid arthritis, anticytoplasmic antibodies	Arthritis Rheum. 2016 Nov;68(11):2778-2783.
AMA-M2 (Mitochondria M2)	With a positive rate of 14.8% in scleroderma, an indicator of concomitant primary biliary cirrhosis (PBC)	Kanzo, Vol.48 No.5 210-218(2007)
p80-coilin	Positive rates of primary biliary cirrhosis (PBC)_3.3% and Sjögren's syndrome_3.7% across autoimmune diseases.	J Exp Med. 1991 Jun 1;173(6):1407-19.
U1-RNP_70	2-14% positive rate for MCTD, DM/PM or SLE overlap syndrome, sausage-like finger inflation, localized skin sclerosis, Raynaud's phenomenon, arthritis, reflux esophagitis is common.	Japanese Journal of Clinical Immunology, 36(3) 139-147(2013)
U1-RNP_ A		
U1-RNP_C		
U2-RNP	Polymyositis-scleroderma overlap syndrome with less than 5% positivity, muscle weakness, and mild scleroderma	Clinical Rheumatology and Related Research, 25:149~158,2013
Ku	Polymyositis-scleroderma overlap syndrome with 2-30% positivity, muscle weakness, minimal skin sclerosis, and good vital prognosis	Clinical Rheumatology and Related Research, 25:149~158,2013
PM-Scl100	2% positive rate in scleroderma, localized skin sclerosis, less frequent serious visceral complications	Japanese Journal of Clinical Immunology, 36(3)139-147(2013)
PM-Scl75		
RuvBL1/2	A positive rate of 1-2% in scleroderma, Overlap of myositis and diffuse skin thickening occur frequently, higher incidence in male and elderly patients	Arthritis Care Res(Hoboken). 2014 Apr;66(4):575-84.
Ki	10% positivity rate in overlap syndromes according to each collagen disease	Laboratory Medicine Encyclopedia & Dictionary
SS-A/Ro52	10-30% positivity in scleroderma	Laboratory Medicine Encyclopedia & Dictionary
SS-A/Ro60		
SS-B	Positive rate of 5% or less in scleroderma	Laboratory Medicine Encyclopedia & Dictionary

Dermatomyositis/Polymyositis Related Antibodies Detection Kit (DM/PM)

In dermatomyositis/polymyositis (hereafter myositis), autoantibodies against various cellular components are detected at a high rate. Among them, myositis-specific autoantibodies are clinically useful, such as diagnosing, classifying disease types, estimating prognosis, and determining treatment. Moreover, many of these corresponding antigens have been elucidated as enzymes and regulators involved in important vital phenomena such as gene transcription/translation and DNA repair, which provide important information for considering the mechanism of autoantibody production and the pathogenesis of myositis.
Eight anti-ARS antibodies have been reported that are specific to myositis and autoantibodies to aminoacyl tRNA synthetase, with common clinical manifestations such as interstitial pneumonitis, fever, arthritis, Raynaud's disease, and mechanics hands. In this kit, all eight types of Jo-1, PL-7, PL-12, EJ, KS, OJ, Zo, Ha can be detected. Especially for anti-OJ antibodies, other than the major antigen, IARS, 10 other antigens that are complexed in vivo can also be detected. To date, there are no assay reagents that can detect antibodies against these at one time, and this reagent enables detection equivalent to immunoprecipitation.
Other myositis-specific antibodies include anti-SRP antibodies, which are correlated with immune-mediated necrotizing myopathy; anti-Mi-2 antibodies, which have high serum CK levels and often have a typical clinical form of dermatomyositis; and anti-TIF1 antibodies (γ, α, and β) and anti-MJ antibodies (NXP-2), which are correlated with malignancy in adults.
In addition, antibodies against SMNs that interact with snRNP, which have been reported as novel myositis-specific antibodies, can also be detected. Myositis-related antibodies include anti-U1RNP antibodies (70, A, and C), anti-U2RNP antibodies, anti-Ku antibodies, anti-PM-Scl antibodies (100,75), anti-RuvBL1/2 antibodies, and anti-Ki antibodies, which are detected by overlap with systemic scleroderma.

Performance correlation data

Percent agreement with samples determined by immunoprecipitation (mean of three autoantibodies each).

- Anti-aminoacyl tRNA synthase (ARSs) Abs (Jo-1, PL-7, PL-12, EJ, KS, OJ)
- Anti-SRP Ab
- Anti-SAE Abs
- Anti-TIF1-alpha/gamma Abs
- Anti-TIF1-β-Abs
- Anti-NXP-2 Ab
- Anti-Mi-2 Ab
- Anti-SS-A Ab
- Anti-SS-B Ab

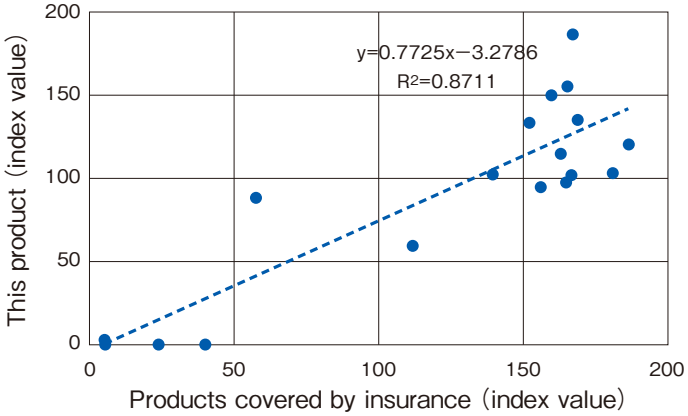
Concordance rate_98% (41/42)

*Discordance : 1 specimen tested positive for anti-TIF1β antibodies by immunoprecipitation → negative by this product

Concordance rate with insurance-listed kits

▶Anti-ARS Ab

26 samples
Correlation coefficient r=0.933



		ELISA (Insurance listed items)	
		+	−
A-Cube	+	14	0
	±	0	0
	−	1	11

Concordance rate_96%

*Since the comparator uses a mixture antigen against Jo-1, PL-7, PL-12, EJ, KS, product also produces and compares mixture antigens for this comparison.

Reference

Dermatomyositis/Polymyositis Related Antibodies Detection Kit (DM/PM)

Antibody	Contents of the report	Reference
ARS	15–20% positivity for myositis, interstitial pneumonia, polyarthritis, Raynaud's phenomenon, fever, mechanics hands are more frequent	Clinical Rheumatology and Related Research, 25:149~158,2013
Jo-1	15–20% positivity for myositis, polymyositis > dermatomyositis	Clinical Rheumatology and Related Research, 25:149~158,2013
PL-7	Less than 5% positivity in myositis	Clinical Rheumatology and Related Research, 25:149~158,2013
PL-12	Positive rate of less than 5% in myositis, interstitial pneumonia > myositis	Clinical Rheumatology and Related Research, 25:149~158,2013
EJ	5–10% positivity for myositis, dermatomyositis > polymyositis	Clinical Rheumatology and Related Research, 25:149~158,2013
KS	Positive rate of less than 5% in myositis, interstitial pneumonia > myositis	Clinical Rheumatology and Related Research, 25:149~158,2013
OJ	Less than 5% positivity in myositis	Clinical Rheumatology and Related Research, 25:149~158,2013
Zo	Less than 1% positivity in myositis	Clinical Rheumatology and Related Research, 25:149~158,2013
Ha	Less than 1% positivity in myositis	Clinical Rheumatology and Related Research, 25:149~158,2013
SRP	Polymyositis with a positive rate of 5%, severe/refractory/ recurrent/necrotizing myopathy, malignancy, and other collagen diseases are less common	Clinical Rheumatology and Related Research, 25:149~158,2013
Mi-2	5–10% positivity in dermatomyositis, low frequency of interstitial pneumonia and arthritis, and good steroid response	Clinical Rheumatology and Related Research, 25:149~158,2013
TIF1-γ (p155)	20% positivity in dermatomyositis, significantly more frequently complicated with malignancy	Clinical Rheumatology and Related Research, 25:149~158,2013
TIF1-α (p140)	10% positive rate in dermatomyositis, significantly more frequently complicated with malignancy	Arthritis Rheum.2012 Feb;64(2):513-22.
TIF1-β	1.5% positivity in dermatomyositis	Arthritis Rheum.2012 Feb;64(2):513-22.
MJ (NXP-2)	Less than 5% positivity for dermatomyositis, and 27–32% positivity for children	Clinical Rheumatology and Related Research, 25:149~158,2013
SAE	Positive rate of less than 1% in dermatomyositis, dysphagia	Clinical Rheumatology and Related Research, 25:149~158,2013
SMN	2.7% positivity in myositis, Ag contributes to snRNPs assembly	Arthritis Rheum.2011 Jul;63(7):1972-8.
cN1A	50% positivity for inclusion body myositis	Front Immunol.2019 Apr 9;10:745.
U1-RNP_70	10% positivity for MCTD, SSc or SLE overlap syndrome, good steroid response, interstitial pneumonia is less common	Clinical Rheumatology and Related Research, 25:149~158,2013
U1-RNP_A		
U1-RNP_C		
U2-RNP	Polymyositis-scleroderma overlap syndrome with less than 5% positivity, muscle weakness, and mild scleroderma	Clinical Rheumatology and Related Research, 25:149~158,2013
Ku	Polymyositis-scleroderma overlap syndrome with 2–30% positivity, muscle weakness, minimal skin sclerosis, and good vital prognosis	Clinical Rheumatology and Related Research, 25:149~158,2013
PM-Scl100	8–10% positivity in polymyositis-scleroderma overlap syndrome, mild	Clinical Rheumatology and Related Research, 25:149~158,2013
PM-Scl75		
RuvBL1/2	A positive rate of 1–2% in scleroderma, Overlap of myositis and diffuse skin thickening occur frequently, higher incidence in male and elderly patients	Arthritis Care Res(Hoboken). 2014 Apr;66(4):575-84.
Ki	10% positivity rate in overlap syndromes according to each collagen disease.	Laboratory Medicine Encyclopedia & Dictionary
SS-A/Ro52	10–20% positivity for myositis	Laboratory Medicine Encyclopedia & Dictionary
SS-A/Ro60		
SS-B	Positive rate of 5% or less in myositis	Laboratory Medicine Encyclopedia & Dictionary

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