IIFT: ANA Mosaic 1 EUROPattern
Instructions for the indirect immunofluorescence test

ORDER NO.	ANTIBODIES AGAINST	SUBSTRATE	SPECIES	FORMAT SLIDES x FIELDS
FC 1510-1005-1 FC 1510-1010-1 FC 1510-2005-1 FC 1510-2010-1	cell nuclei (ANA global test)	HEp-2 cells liver (2 BIOCHIPs per field)	human monkey	10 x 05 (050) 10 x 10 (100) 20 x 05 (100) 20 x 10 (200)

Indication: Many autoimmune diseases, particulary of the rheumatic form.

Application: Indirect immunofluorescence (IIFT) is the gold standard for the determination of antibodies against nuclear antigens (ANA). The EUROPattern test systems have been developed specifically for the high-performance automation solution from EUROIMMUN AG.

Test principle: This test kit is designed exclusively for the in vitro determination of human antibodies in patient samples. The determination can be performed qualitatively or quantitatively.

Substrate combinations of HEp-2 cells and primate liver are incubated with diluted patient sample. If a positive reaction is obtained, specific antibodies of classes IgA, IgG and IgM attach to the antigens. In a second step, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and made visible with the fluorescence microscope.

Contents of a test system for 50 determinations: FC 1510-1005-1 (IgG)

Description	Format	Symbol
1. Slides, each containing 5 x 2 BIOCHIPs coated with HEp-2 cells and primate liver	10 slides	SLIDE
2. Fluorescein-labelled anti-human IgG (goat), with propidium iodide for EUROPattern, ready for use	1 x 1.5 ml	CONJUGATE PI
3. Positive control: autoantibodies against cell nuclei (ANA), for EUROPattern, human, ready for use	1 x 0.25 ml	POS CONTROL
4. Negative control: autoantibody-negative, human, ready for use	1 x 0.25 ml	NEG CONTROL
5. Salt for PBS pH 7.2	2 packs	PBS
6. Tween 20	2 x 2.0 ml	TWEEN 20
7. Embedding medium, ready for use	1 x 3.0 ml	GLYCEROL
8. Cover glasses (62 mm x 23 mm)	12 pieces	COVERGLASS
9. Instruction booklet	1 booklet	---

LOT	Lot description		Storage temperature
IVD	In vitro diagnostics	CE	Unopened usable until

Single slides (e.g., EUROIMMUN order no. FW 1510-1005-1) are provided together with cover glasses. Additional positive control (e.g., order no. CA 1570-0102-4) and negative control (e.g., order no. CA 1000-0102) can be ordered.
Performance of the test requires reagent trays TRAY, which are not provided in the test kits. They are available from EUROIMMUN under the following order no.:
- ZZ 9999-0110 Reagent trays for slides containing up to 10 fields

Storage and stability: The slides and the reagents should be stored at a temperature between +2°C and +8°C. Stability is guaranteed for 18 months after the date of manufacture if stored properly.

Waste disposal: Patient samples, controls and slides are to be handled as potentially infectious materials. All reagents are to be disposed of in accordance with official disposal regulations.

Performing the test (reaction fields 5 x 5 mm)

The **TITERPLANE Technique** was developed by EUROIMMUN in order to standardize immunological analyses: Samples or labelled antibodies are applied to the reaction fields of a reagent tray. The BIOCHIP slides are then placed into the recesses of the reagent tray, where all BIOCHIPs of the slide come into contact with the fluids, and the individual reactions commence simultaneously. Position and height of the droplets are exactly defined by the geometry of the system. As the fluids are confined to a closed space, there is no need to use a conventional "humidity chamber". It is possible to incubate any number of samples next to each other and simultaneously under identical conditions.

Prepare: The preparation of the reagents and of the serum and plasma samples is described on **page 4** of this test instruction.

Pipette: Apply **30 µl of diluted sample** to each reaction field of the reagent tray, avoiding air bubbles. Transfer all samples to be tested before starting the incubation (up to 200 droplets). Use a polystyrene pipetting template.

Incubate: Start reactions by fitting the BIOCHIP slides into the corresponding recesses of the reagent tray. Ensure that each sample makes contact with its BIOCHIP and that the individual samples do not come into contact with each other. Incubate for **30 min** at room temperature (+18°C to +25°C).

Wash: Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and immerse them immediately afterwards in a cuvette containing PBS-Tween for at least **5 min**. Shake with a rotary shaker if available. Wash max. 16 slides then replace PBS-Tween with new buffer.

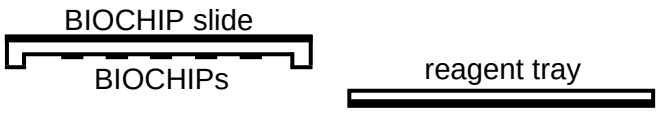
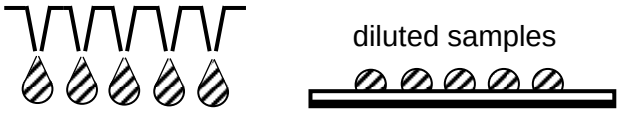
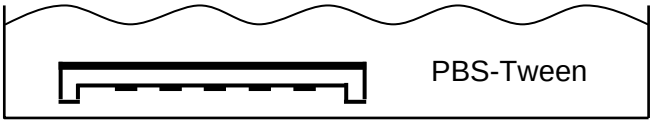
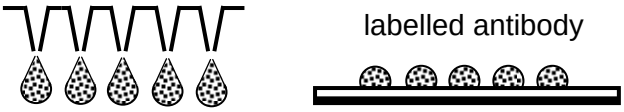
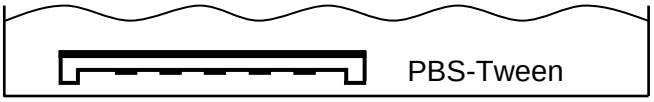
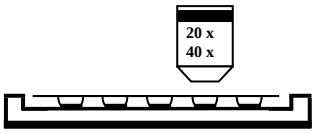
Pipette: Apply **25 µl of fluorescein labelled anti-human globulin** to each reaction field of a clean reagent tray. Add all droplets before continuing incubation. Use a stepper pipette. The labelled anti-human serum should be mixed thoroughly before use. To save time, conjugate can be pipetted onto separate reagent trays during the incubation with the diluted sample.

Incubate: Remove one BIOCHIP slide from cuvette. Within five seconds blot only the back and the long sides with a paper towel and immediately put the BIOCHIP slide into the recesses of the reagent tray. Do not dry the areas between the reaction fields. Check for correct contact between the BIOCHIPs and liquids. Then continue with the next BIOCHIP Slide. From now on, protect the slides from direct sunlight. Incubate for **30 min** at room temperature (+18°C to +25°C).

Wash: Fill cuvette with new PBS-Tween. Rinse the BIOCHIP slides with a flush of PBS-Tween using a beaker and put them into the cuvette filled with the new PBS-Tween for at least **5 min**. Shake with a rotary shaker if available. Wash max. 16 slides then replace PBS-Tween with new buffer.

Embed: Place embedding medium onto a cover glass - drops of **max. 10 µl per reaction field**. Use a polystyrene embedding template. Remove one BIOCHIP slide from PBS Tween and dry the back, all four sides, as well as the surface around, but not between the reaction fields with a paper towel. Put the BIOCHIP slide, with the BIOCHIPs facing downwards, onto the prepared cover glass. Check immediately that the cover glass is properly fitted into the recesses of the slide. Correct the position if necessary.

Evaluate: Read the fluorescence.
General recommendation: objective 20x (tissue sections, infected and transfected cells), 40x (cell substrates).
EUROPattern: excitation filter: 450-490 nm, colour separator: 510 nm, long-pass filter: 515-565 nm.
Visual examination: excitation filter: 450-490 nm, colour separator: 510 nm, blocking filter: 515 nm
Light source: mercury vapour lamp, 100 W, EUROIMMUN LED, EUROStar Bluelight.

TITERPLANE Technique		
Pipette:	30 µl per field	
Incubate:	30 min	
Wash:	1 s flush 5 min cuvette	
Pipette:	25 µl per field	
Incubate:	30 min	
Wash:	1 s flush 5 min cuvette	
Embed:	max. 10 µl per field	
Evaluate:	EUROPattern microscopy	

Preparation and stability of reagents

Note: Unless stated otherwise, after initial opening the reagents are stable until the expiry date, when stored between +2°C and 8°C and protected from contamination.

- **Slides:** Ready for use. Remove the protective cover only when the slides have reached room temperature (condensed water can damage the substrate). Mark with a felt-tip pen. Do not touch the BIOCHIPs. If the protective cover is damaged, the slide must not be used for diagnostics.

- **Fluorescein-labelled secondary antibody (FITC):** Ready for use. Before using for the first time, mix thoroughly. The conjugate is sensitive to light. Protect from sunlight.

- **Positive and negative controls:** Ready for use. Before using for the first time, mix thoroughly.

- **PBS-Tween:** 1 pack of “Salt for PBS” should be dissolved in 1 liter of distilled water (optimal: aqua pro infusione, aqua ad injectabilia) and mixed with 2 ml of Tween 20 (stir for 20 min until homogeneous). The prepared PBS-Tween can be stored at +2°C to +8°C, generally for 1 week. PBS-Tween should not be used if the solution becomes cloudy or contamination appears.

- **Embedding medium:** Ready for use.

- **Reagent trays:** Reaction fields of the reagent tray must be hydrophilic and surrounding area hydrophobic. If necessary, wipe with Extran MA 01 (EUROIMMUN order no. ZZ 9911-0130) and rinse generously with water. To disinfect: Immerse in Sekusept Extra (Henkel) (3% in water) for 1 hour. After disinfection rinse generously with water and dry with absorbent paper.

Warning: The BIOCHIPs coated with antigen substrates have been treated with a disinfecting fixing agent. Neither HBsAg nor antibodies against HIV-1, HIV-2, and HCV could be detected in the control sera using appropriate ELISA or indirect immunofluorescence tests**. Nevertheless, all test system components should be handled as potentially infectious materials.** Some of the reagents also contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of serum and plasma samples

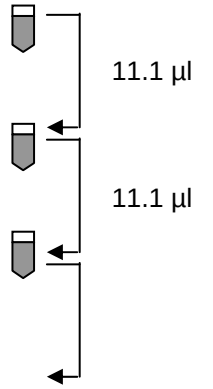
Samples: Human sera or EDTA, heparin or citrate plasma.

Stability: The **patient samples** to be investigated can generally be stored up to 14 days at a temperature between +2°C and +8°C. Diluted samples must be incubated within one working day.

Recommended sample dilution for qualitative evaluation: The sample to be investigated is diluted 1:100 in PBS-Tween. For example, dilute 10.1 µl sample in 1000 µl PBS-Tween and mix thoroughly, e.g., vortex for 4 seconds.

Recommended sample dilution for quantitative evaluation: The dilution of samples to be investigated is performed using PBS-Tween. For each add 100 µl of PBS-Tween to the tube and mix with 11.1 µl of the next highest concentration, e.g. vortex for 2 seconds. EUROIMMUN recommends incubating samples from a dilution of 1:100.

Dilution	Dilution scheme
1:10	100 µl PBS-Tween + 11.1 µl undiluted sample
1:100	100 µl PBS-Tween + 11.1 µl 1:10 diluted sample
1:1000	100 µl PBS-Tween + 11.1 µl 1:100 diluted sample
⋮	⋮



After every two dilution steps, a new pipette tip should be used to prevent carryover.

Evaluation

Fluorescence pattern (positive reaction): The fluorescence is evaluated using EUROPattern. Antibodies against nuclear antigens (ANA) can be found on numerous substrates. For the targeted determination and differentiation of antinuclear antibodies, a substrate consisting of human epithelial cells (HEp-2) and primate liver is suitable. The cell nuclei show a distinct fluorescence, which is characterized by certain patterns. In the case of negative samples, the nuclei show no specific fluorescence. In each field evaluated, both interphase nuclei and mitotic cells of the HEp-2 cells as well as the liver should be examined, and this in several areas if possible.

If the positive control shows no specific fluorescence pattern or the negative control shows a clear specific fluorescence, the results are not to be used and the test is to be repeated.

A large range of fluorescence images can be found on the EUROIMMUN website (www.euroimmun.com)

Qualitative evaluation:

ANA reactivity	**Evaluation**
No reaction at 1:100	Negative. No antibodies against cell nuclei detectable in the patient samples
Positive reaction at 1:100	Trace. For IF types: pattern homogeneous, centromeres, nuclear dots, Jo-1, typical patterns of SS-A/SS-B, Sm/RNP possible indication of various rheumatic and other diseases.
Positive reaction at 1:320	Positive. Indication of various rheumatic and other diseases.

Quantitative evaluation: The titer is defined as the sample dilution factor for which specific fluorescence is just identifiable. This should be compared to the reaction obtained with an equivalently diluted negative serum.

Antibody titers can be determined according to the following table from the fluorescence of the different sample dilutions.

Fluorescence at				Antibody titer
1:10	1:100	1:1000	1:10000	
weak	negative	negative	negative	1:10
moderate	negative	negative	negative	1:32
strong	weak	negative	negative	1:100
strong	moderate	negative	negative	1:320
strong	strong	weak	negative	1:1000
strong	strong	moderate	negative	1:3200
strong	strong	strong	weak	1:10000
⋮	⋮	⋮	⋮	⋮

For diagnosis the clinical symptoms of the patient should always be taken into account along with the serological results.

Some of the most important fluorescence patterns are the homogeneous and granular nuclear fluorescence as well as staining of the nucleoli and the centromeres (clearly identifiable especially in the mitotic cells). The relevant binding patterns frequently correspond with biochemically defined nuclear antigens:

Autoantigens of the cell nuclei		**Fluorescence pattern**
Polynucleotides	Double-stranded DNA	Homogeneous
	Single-stranded DNA	Homogeneous
	RNA	Partly homogeneous
Histones	H1, H2A, H2B, H3, H4, H2A-H2B complex	Homogeneous
Ribonucleoproteins of the nucleoplasm (ENA)	U1-nRNP	Coarse granular, nulceoli negative
	Sm	Coarse granular, nulceoli negative
	SS-A (Ro)	Granular
	SS-B (La)	Granular
Antigens of the nucleolus	U3-nRNP/fibrillarin	Nucleoli, granular
	RNA polymerase I	Nucleoli, granular
	PM-Scl (PM-1)	Nucleoli, homogeneous
	7-2-RNP (To)	Nucleoli, homogeneous
	4-6-S-RNA	Nucleoli, homogeneous
Centromeres	Proteins of kinetochores	Typical granular
Other proteins	Scl-70	Nearly homogeneous, marked nucleoli
	Cyclin (PCNA)	Granular, 50% 10 times brighter
	Nuclear dots	Nuclear dots
	NOR-90	Metaphase 1-2 granula
	Ku	Reticular
	Mi-1	Fine granular
	Mi-2	Fine granular
	Lamins	Nuclear membrane

If each sample is investigated with a substrate combination of HEp-2 cells and primate liver (Order No. FC 1510-1005-1) a comparison of the fluorescence patterns allows cell nuclei antibodies to be further differentiated: antibodies against U1-nRNP, Sm, dsDNA, histones and nuclear dots react with HEp-2 cells and liver with the same intensity, whereas antibodies against SS-A, SS-B, centromeres and cyclin show a much weaker fluorescence pattern on liver tissue.
Antibodies against Ku show only a granular fluorescence on the HEp-2 cells, whereas a coarse granular, perinuclear fluorescence can be seen on primate liver, which can be clearly differentiated from other fluorescence patterns. Only with the HEp-2 cells and primate liver combination is it possible to determine among other things if antibodies against centromeres and nuclear dots are present in the same sample.

Test characteristics

Antigen: For the detection of antinuclear antibodies by indirect immunofluorescence, patient sera are nowadays predominantly tested using a combination of two substrates: human epithelial cells (HEp-2) and frozen sections of primate liver. Comparing the fluorescence patterns makes the pre-differentiation of a large number of antibodies possible. The primate liver completes the spectrum of detectable autoantibodies. Important are antibodies against ribosomal P-proteins, liver cell membrane (LMA), bile ducts, endothelial cells, endomysium and granulocytes (cANCA, pANCA).

If the indirect immunofluorescence test is positive, it can be followed up by various monospecific analyses (e.g., enzyme immunoassays) in order to identify the specific antinuclear antigens against which the antibodies are directed.

Stability: Stability is guaranteed for 18 months after the date of manufacture if stored properly.

Measurement range: The dilution starting point for this measurement system is 1:100. Samples can be further diluted by a factor of 10 so that the dilution series is 1:1000, 1:10000 etc. There is no upper limit to the measurement range.

Intra-assay reproducibility: Inter-lot reproducibility was tested with more than 10 different lots. In quantitative evaluation of results, the deviation amounted to no more than ± 1 fluorescence intensity level for all samples. Intra-assay reproducibility can therefore also be guaranteed. The intensity of the specific fluorescence as a numeric value is called fluorescence intensity level by EUROIMMUN. These values can reach from “0“ (no specific fluorescence) to “5“ (extremely strong specific fluorescence).

Inter-assay reproducibility: Inter-lot reproducibility was tested with more than 10 different lots. In quantitative evaluation of results, the deviation amounted to no more than ± 1 fluorescence intensity level for all samples. Inter-assay reproducibility can therefore also be guaranteed.

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Cross reactivity: 11 characterized samples were incubated, which did not produce any unexpected fluorescence pattern (CDC 1 to CDC 11). The results showed no cross reactivity with these sera.

Interference: Hemolytic, lipemic and icteric samples showed no interferences on analysis results.

Reference range: Titer 1: < 100
The following antibody prevalences were determined using a panel of samples from healthy blood donors (origin of samples: Germany).

Substrate	Antibodies against	Conjugate	Prevalence	Cut-off	Number of samples
Hep-2 cells (human)	ANA	IgG	12.5%	1:100	n = 200
Liver (monkey)			4.5%		

Specificity and sensitivity:

Substrate	Ig class	Reference (number of samples, origin of samples)	Specificity	Sensitivity
HEp-2-cells (human): ANA	IgG	ELISA + Blot (n = 128, Germany)	100%	100%
Liver (monkey): ANA	IgG	IIFT: HEp-2 (n = 263, Germany)	97%	81%

HEp-2 EUROPattern (software validation):

Visual evaluation		Total (n = 351)	
		Positive	Negative
EUROPattern	Positive	272	2
	Negative	0	77
	Concordance	99.4%	
	Sensitivity	100%	
	Specificity	97.5%	

Comparison of software-based and visual positive/negative classification

EUROPattern result	Total (n = 351)	
Main ANA pattern	**No. of samples**	**Pattern recognized**
Homogenous	33	27 (81,8 %)
Speckled	130	123 (94,6 %)
Nucleolar	45	43 (95,6 %)
Centromeres	4	4 (100 %)
Nuclear dots	2	2 (100 %)
Cytoplasmic	58	54 (93,1 %)
Negative	79	77 (97,5 %)
Total	**351**	**330 (94,0 %)**

Main pattern recognition by EUROPattern

Clinical significance

The detection of autoantibodies against cell nuclei (ANA) is an important diagnostic indicator in many autoimmune diseases. Antibodies against nuclear antigens are directed against various cell nuclear components (biochemical substances in the cell nucleus). These encompass nucleic acids, cell nucleus proteins and ribonucleoproteins. They are a characteristic finding in many diseases, in particular rheumatic diseases. The frequency (prevalence) of anti-nuclear antibodies in inflammatory rheumatic diseases is between 20% and 100%, the lowest occurring in rheumatoid arthritis at between 20% and 40%. Therefore, differential antibody diagnostics against nuclear antigens is indispensible for the identification of individual rheumatic diseases and their differentiation from other autoimmune diseases:

Autoimmune disease	Prevalence ANA
Systemic lupus erythematosus (SLE)	80% - 100%
Drug-induced erythematosus	100%
Mixed connective tissue disease (MCTD, Sharp syndrome) Rheumatoid arthritis Other rheumatic diseases	100% 20% - 40% 20% - 50%
Progressive systemic sclerosis Polymyositis and dermatomyositis Sjögren's syndrome	85% - 95% 30% - 50% 70% - 80%
Autoimmune hepatitis	30% - 40%
Ulcerative colitis	26%

Systemic lupus erythematosus

For systemic lupus erythematosus (SLE), the determination of antibodies against double-stranded DNA is considered to be one of the most important criteria for diagnosis. Immune complexes of double-stranded DNA and the corresponding autoantibodies cause tissue damage in the subcutis, the kidneys and other organs. The antibody titer correlates with the clinical activity of the disease. Autoantibodies against nucleosomes occur independently of anti-dsDNA antibodies; 18% of SLE sera react exclusively with nucleosomes and not with dsDNA. Antibodies against nucleosomes generally indicate a severe course of SLE. They occur at a prevalence of 9%-89% in cases without lupus nephritis and at a prevalence of 18%-86% in cases with lupus nephritis. Furthermore, antibodies against Sm are also considered to be pathognomonic for SLE. In addition, antibodies against other polynucleotides, ribonucleotides, histones and other nuclear antigens can be found.

In drug-induced lupus erythematosus with manifestations such as arthralgia, arthritis, exanthema, serositis, myalgia, heptomegalia and splenomegalia, antibodies against histones are observed. This reversible form of SLE can be induced by antibiotics (e.g. penicillin, streptomycin, tetracyclines), chemotherapeutic agents (e.g. INH, sulfonamides), anticonvulsants (e.g. phenytoin, hydantoins), antiarrythmics (e.g. procainamide, practolol), antihypertensives (e.g. reserpine, hydralazine), psychotropics (e.g. chlorpromazine), antithyroid drugs (e.g. thiouracil derivates), antirheumatoid basic therapeutics (e.g. gold, D-penicillamine) and other drugs such as contraceptives and allopurinol.

Autoantibodies in systemic lupus erythematosus	
Antigen	Prevalence
Double-stranded DNA	60% - 90%
Single-stranded DNA	70% - 95%
RNA	50%
RNA helicase A	6%
Histones	50% - 80%
Nucleosomes, dependent on SLE severity either without or with lupus nephritis	9% - 89% or 18% - 86%
U1-nRNP	15% - 40%
Sm	5% - 40%
SS-A (Ro)	20% - 60%
SS-B (La)	10% - 20%
Cyclin (PCNA)	3%

Ku	10%
Ribosomal P-proteins	10%
(Hsp-90: Heat shock protein, 90 kDA	50%**)**
(Cardiolipin	40% - 60%**)**

Sharp syndrome

High autoantibody titers against U1-nRNP are characteristic for Sharp syndrome (MCTD = Mixed connective tissue disease). The antibody titer correlates with the clinical activity of the disease.

Autoantibodies in Sharp syndrome (Mixed connective tissue disease = MCTD)	
Antigen	Prevalence
U1-nRNP	95% - 100%
Single-stranded DNA	20% - 50%

Rheumatoid arthritis

In rheumatoid arthritis, antibodies against histones can be observed in more than half of all cases, while antibodies against U1-nRNP are rarely found. Antibodies against RANA ("rheumatoid arthritis nuclear antigen") cannot be detected with HEp-2 cells.

Cell nuclei antibodies in rheumatoid arthritis	
Antigen	Prevalence
Histones	15% - 50%
Single-stranded DNA	8%
U1-nRNP	3%
(RANA	90% - 95%**)**

Progressive systemic sclerosis

Progressive systemic sclerosis (PSS; scleroderma) can manifest itself in two forms, which cannot always be clearly differentiated. Until now, antibodies against fibrillarin, RNA-polymerase I and Scl-70 have only been noticed in the diffuse form. Autoantibodies against centromeres are associated with the limited form of progressive systemic sclerosis.

Autoantibodies in progressive systemic sclerosis (diffuse form)	
Antigen	Prevalence
Fibrillarin	5% - 10%
PM-Scl (PM-1), including overlap syndrome	50% - 70%
Scl-70	25% - 75%
RNA polymerase I	4%
7-2-RNP (To)	rare
NOR-90 (nucleolus organiser region)	rare

Autoantibodies in progressive systemic sclerosis (limited form)	
Antigen	Prevalence
Centromeres	80% - 95%

Polymyositis / dermatomyositis

Autoantibodies against PM-Scl occur in polymyositis and dermatomyositis, but other nuclear antibodies (against Mi-1, Mi-2, Ku) and antibodies against Jo-1 can also be identified in these diseases .

Autoantibodies in polymyositis and dermatomyositis	
Antigen	Prevalence
PM-Scl (PM-1), including overlap syndrome	50% - 70%
Jo-1 (histidyl-tRNA synthetase)	25% - 35%
Mi-1	10%
Mi-2	5%
Ku	50%
Single-stranded DNA	40% - 50%
PL-7 (threonyl-tRNA synthetase)	4%
PL-12 (alanyl-tRNA synthetase)	3%

Sjögren's syndrome

In Sjögren's syndrome (primary Sjögren's syndrome), antibodies against SS-A and SS-B are present, mainly in combination with one another. In addition, autoantibodies against the secretory ducts of the salivary gland are found in 40 to 60% of all cases.

Autoantibodies in primary Sjögren´s syndrome	
Antigen	Prevalence
SS-A (Ro)	40% - 95%
SS-B (La)	40% - 95%
Single-stranded DNA	13%
(RANA	70%**)**
(Salivary gland excretory ducts	40% - 60%**)**
(Rheumatoid factors	60% - 80%**)**

Autoantibodies against nuclear antigens occur in many other diseases, such **as primary biliary liver cirrhosis** ("Nuclear Dots", SS-A) and **chronic active autoimmune hepatitis** (SS-A, lamins). At times, antibodies against nuclear antigens are detectable in subjectively healthy individuals, usually at a low titer (various immunoglobulin classes, mainly IgM).

Autoantibodies against cell nuclei: Important associated diseases		
Antigen	Disease	Prevalence
Double-stranded DNA	Systemic lupus erythematosus (SLE)	60% - 90%
Single-stranded DNA	Systemic lupus erythematosus (SLE) Drug-induced erythematosus Mixed connective tissue disease (MCTD or Sharp syndrome) Polymyositis / dermatomyositis Scleroderma, Sjörgren's syndrome, rheum. arthritis	70% - 95% 60% 20% - 50% 40% - 50% 8% - 14%
RNA	Systemic lupus erythematosus Scleroderma, Sjörgren's syndrome	50% 65%
Histones	Drug-induced lupus erythematosus (SLE) Systemic lupus erythematosus Rheumatoid arthritis	95% 58 % - 80 % 15% - 50%
U1-nRNP	Mixed connective tissue disease (MCTD, Sharp syndrome) Systemic lupus erythematosus Rheumatoid arthritis	95% -100% 15% - 40% 3%
Sm	Systemic lupus erythematosus (SLE)	5% - 40%
Nucleosomes	Systemic lupus erythematosis (SLE) without lupus nephritis with lupus nephritis	 9% - 89% 18% - 86%
SS-A (Ro)	Sjögren's syndrome Systemic lupus erythematosus (SLE) Neonatal lupus syndrome	40% - 95% 20% - 60% 100%
SS-B (La)	Sjögren's syndrome Systemic lupus erythematosus (SLE)	40% - 95% 10% - 20%
Fibrillarin	Progressive systemic sclerosis, diffuse form	5% - 10%
RNA polymerase I	Progressive systemic sclerosis, diffuse form	4%
PM-Scl (PM-1)	Polymyositis/dermatomyositis/overlap syndrome Progressive systemic sclerosis, diffuse form	50% - 70% 5% - 10%
Centromeres	Progressive systemic sclerosis, limited form	80% - 95%
Scl-70	Progressive systemic sclerosis, diffuse form	25% - 75%
Cyclin (PCNA)	Systemic lupus erythematosus (SLE)	3%
Ku	Systemic lupus erythematosus (SLE) Poly-/dermatomyositis, progressive systemic sclerosis	10% 30% - 55%
Mi-1, Mi-2	Dermatomyositis	5% - 10%

Antibodies against components of the cytoplasm of HEp-2 cells cannot always be clearly differentiated. Only a few cytoplasm-reactive antibodies can be assigned to a particular disease, e.g. antibodies against mitochondria in primary biliary liver cirrhosis and against the proteins PL-7 and PL-12 in polymyositis and dermatomyositis. Further rare antibodies found in polymyositis are those directed against OJ, EJ and signal recognition particles (SRP). Other cytoplasmic antibodies — against ribosomes, Golgi apparatus, lysosomes, and cytoskeletal components, such as actin, vimentin and cytokeratins — are of minor clinical significance. The diagnostic value of mitosis-associated antigens has also not yet been finally clarified. All these arguments show the high immunological relevance and the resulting diagnostic value of autoantibodies against cell nuclei (ANA).

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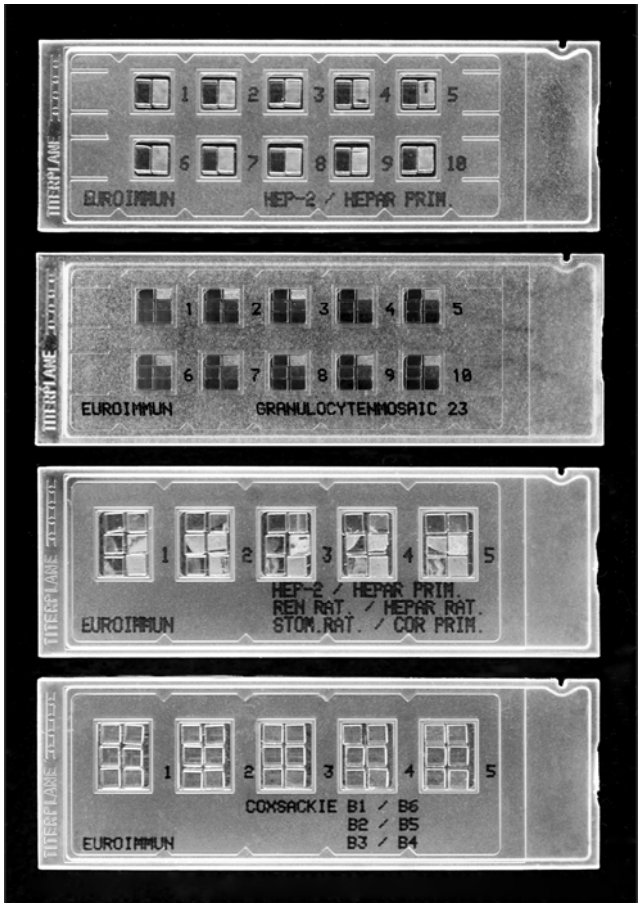

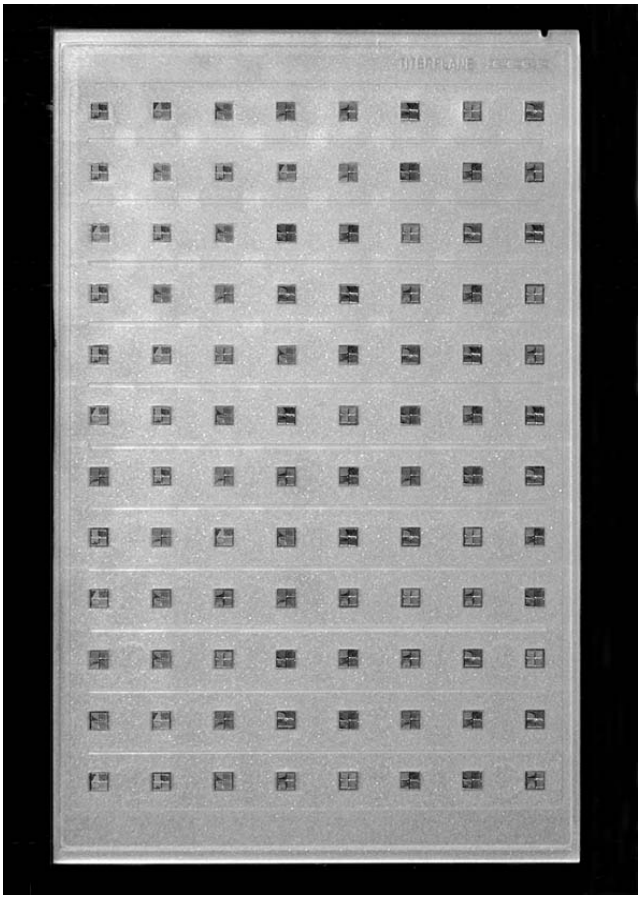

Slides for indirect immunofluorescence can be produced with single substrates or BIOCHIP Mosaics™ from up to 45 different substrates according to your individual requirements. thyroid gland, parathyroid gland, pancreas, adrenal gland, ovary, placenta*, testis, spermatozoa, pituitary gland, hypothalamus*, cerebrum, cerebellum, brain stem, pons*, lobus temporalis, substantia nigra*, peripheral nerve, spinal cord, optic nerve (AQP-4, NMO IgG) eye, granulocytes (fixed with EOH, HCHO or MOH), lactoferrin-specific granulocytes, lymphocytes, monocytes*, thrombocytes, kidney (primate, rat, mouse), lung*, liver (primate, rat, mouse), mouth mucosa, stomach (corpus, antrum), jejunum, colon, intestinal goblet cells, umbilical cord, mamma, lacrimal gland, parotid gland, prostate, vesicula seminalis, skeletal muscle, F-actin (VSM47), heart muscle, thymus, lipocytes, cartilage*, epidermis, oesophagus (primate, rat), tongue, lip, melanocytes, HEp-2 cells, HEp-20-10 cells, HUVEC, Crithidia luciliae sensitive etc. Adenovirus, Afipia felis*, Bartonella henselae, B. quintana, Bordetella parapertussis, B. pertussis, Borrelia afzelii, B. burgdorferi sensu stricto (strains CH, USA), B. garinii, Campylobacter coli*, C. jejuni, Candida albicans, C. glabrata*, C. krusei*, C. parapsilosis*, C. tropicalis*, Chikungunya virus, Chlamydia pneumoniae, C. trachomatis, C. psittaci, CMV, Coxsackievirus (A7, A9, A16, A24, B1 to B6), Crimean Congo fever virus, Dengue virus type 1 to 4, EBV-CA, EBNA, EBV-EA, Echinococcus granulosus, ECHO virus, Hantavirus, Haemophilus influenzae*, Helicobacter pylori, HHV-6, HSV-1, HSV-2, Influenza virus A (strains H3N2, H1N1, H5N1), Influenza virus B, Japanese encephalitis virus, Klebsiella pneumoniae*, Legionella bozemanii*, L. dumoffii*, L. gormanii*, L. jordanis*, L. longbeachae, L. micdadei*, L. pneumophila (serotypes 1 to 14), Leishmania donovani, Listeria monocytogenes (1/2a and 4b)*, measles virus, mumps virus, Mycoplasma hominis, M. pneumoniae, Parainfluenza virus type 1 to 4, Rift valley fever virus*, RSV, rubella virus, Saccharomyces cerevisiae, SARS-CoV, TBE virus, TO.R.C.H. profile, Toxoplasma gondii, Treponema pallidum, T. phagedaenis, Ureaplasma urealyticum, VZV, West Nile virus, Yellow fever virus, Yersinia enterocolitica (O:3, O:4, O:6 and O:9)*. EUROPLUS™: HEp-2/liver + RNP/Sm, Sm, SS-A, SS-B, Scl-70, rib. P-proteins, Jo-1; granulocytes + MPO, PR3; primate stomach (parietal cells) + intrinsic factor; primate liver (endomysium) + gliadin (GAF-3X); rat kidney + AMA M2; thyroid gland + thyroglobulin; Borrelia burgdorferi and afzelii + OspC and VlsE, Plasmodium falciparum (HRP-2, MSP-2), P. vivax (MSP, CSP). Transfected cells: rPAg 1 + 2 (pancreas antigen 1 + 2), AQP-4, glutamate receptor (type NMDA), desmoglein 1 + 3, BP230.
* Currently not available in the European Union.