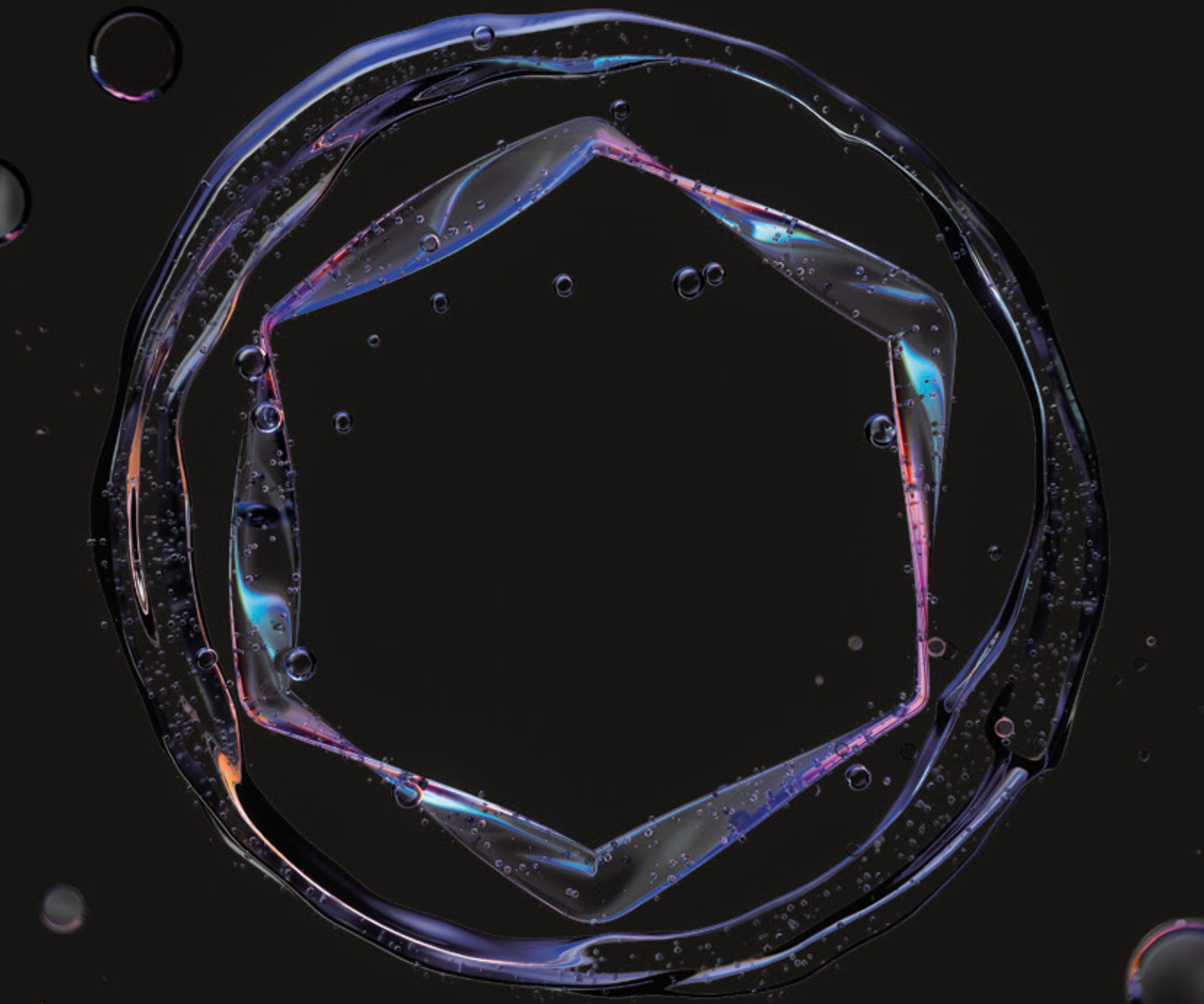
BBI

BBISOLUTIONS.COM

Autoimmunity guidebook



Advancing health, together

For over 70 years, we've specialized in the development and manufacturing of world-class reagents, both native and recombinant, helping IVD and life science teams transform scientific innovation into trusted, life-saving diagnostics.

Across our portfolio, from antibodies, enzymes, and antigens to complex conjugates and custom solutions, every product is developed with precision to support performance, regulatory compliance, and diagnostic accuracy.

But it's not just about the products. We know what's at stake. That's why our scientists work shoulder-to-shoulder with yours, solving challenges, refining performance, and accelerating progress from concept to commercialization. Our continued investment in next-generation recombinant technologies reflects that commitment: enabling you to bring diagnostics and life science solutions to market faster, with greater confidence, resilience, and sustainability.

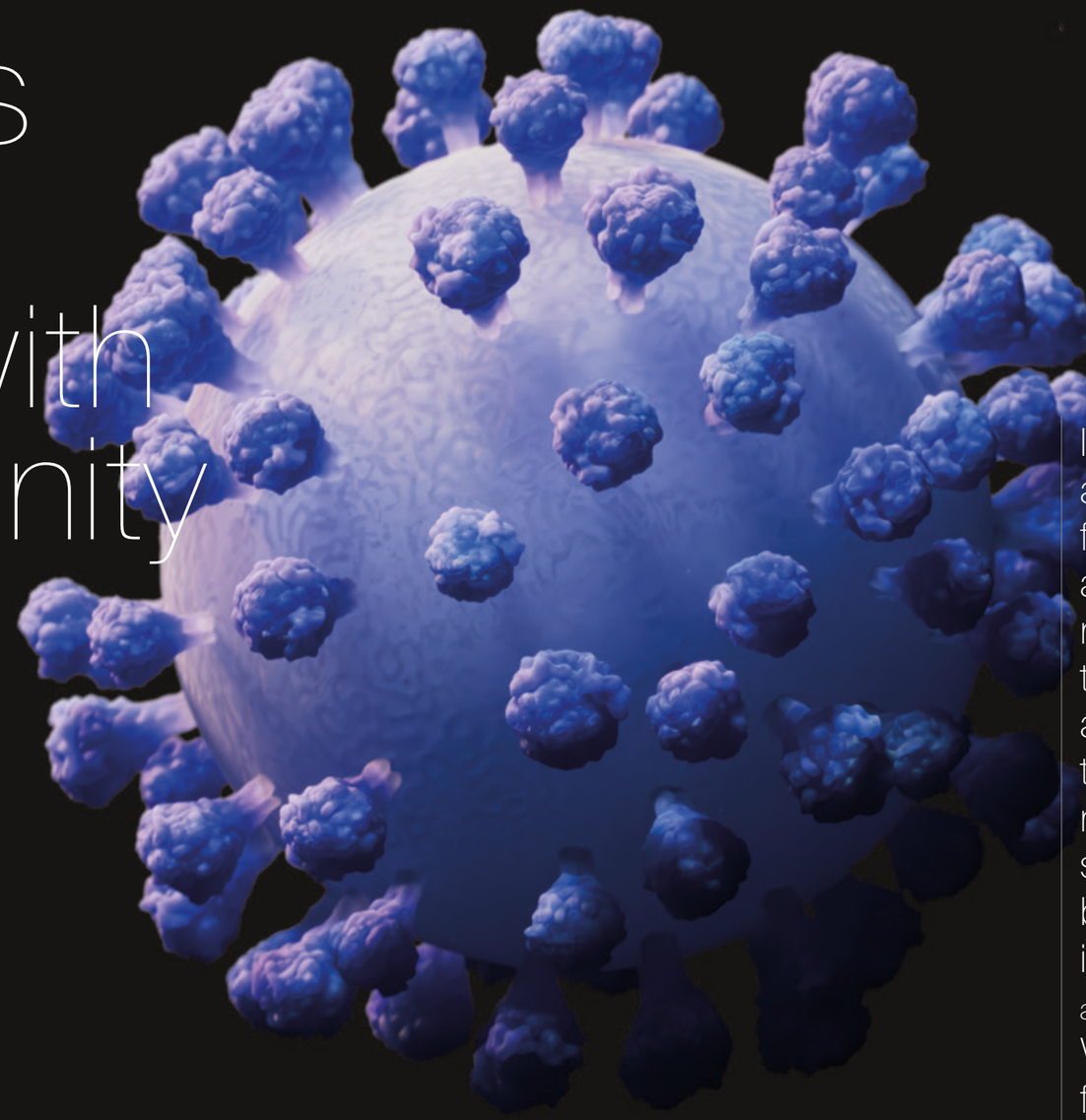
As you explore this brochure, we hope you'll see what sets BBI apart - not just in what we make, but in how we show up as your partner.

Welcome to BBI. Let's advance health, together.

Alex Socarrás
Group CEO

Contents

Modern medicine's growing concern with autoimmunity



01

In healthy individuals the immune system acts as a defensive line to protect us from a variety of infectious agents such as bacteria, toxins and viruses. A key requirement to fulfill this defense is for the body to be able to distinguish 'foreign agents' from 'self', a process that needs to be tightly regulated. When these regulation mechanisms fail, the immune system can attack not only pathogens but also the body's own tissues, resulting in autoimmune diseases (Riedhammer and Weissert, 2015; Mohammadi et al. 2022). Worldwide, millions of people suffer from autoimmune diseases.

This article discusses the discovery, prevalence, healthcare costs, diagnostic tools, and upcoming developments in the field of autoimmune diseases.

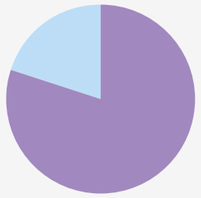
Introduction

The discovery of autoimmune diseases dates to the early 20th century, when doctors first noticed that some patients had symptoms that seemed to be caused by their own immune systems.

However, it wasn't until the 1950s and 1960s that scientists began to fully understand the underlying mechanisms of autoimmunity (Ahsan, 2022). Today, there are more than 80 different autoimmune diseases that have been identified, each with its own unique set of causes. (Rose, 2016).

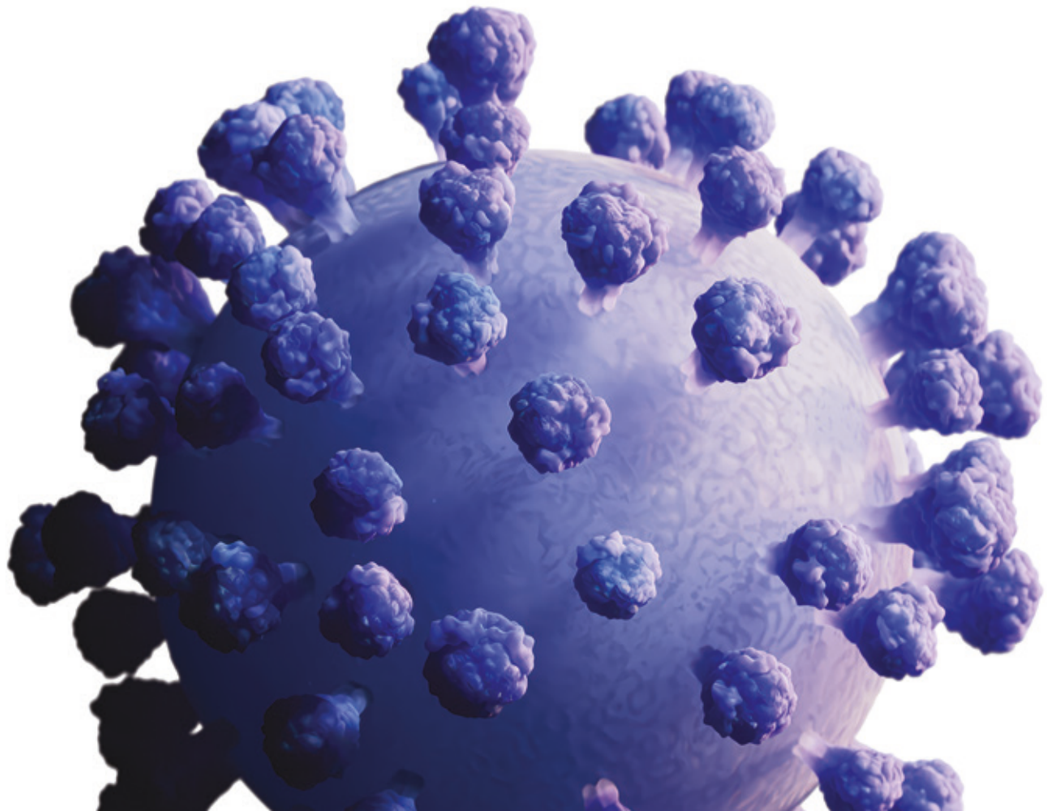
It is estimated that 8% of the population suffers from autoimmunity worldwide. Some of the most common autoimmune diseases include multiple sclerosis, rheumatoid arthritis, and type 1 diabetes. Autoimmune diseases affect more women than men, with approximately 80% of all patients being female, and their risk increases with age. (Whitacre 2001; Fairweather and Rose 2004; Figure 1). In fact, autoimmune diseases are a leading cause of death in women (Walsh and Rau, 2000). In addition, a number of studies have also suggested that autoimmune diseases have become more prevalent in recent decades (Miller, 2022).

Percentage of female vs. male affected by disease type.



80%

of patients diagnosed with autoimmune disease are women.



The healthcare costs associated with autoimmune diseases are spiraling. In the United States alone, it is estimated that the annual cost of treating autoimmune diseases is around $100 billion[1]. This includes the cost of medications, hospital stays, and lost productivity due to missed work.

[1] AARDA report, 2011

Diagnosing autoimmune diseases can be difficult as many systemic autoimmune diseases have similar symptoms, and the underlying causes are often unknown.

However, there are several tools that are commonly used to aid diagnosis, including serological immunoassays, immunofluorescent assays, and biopsies. Autoantibodies alone are not diagnostic for the disease, but they do indicate the presence of autoimmunity. More recently, research has been focused strongly on predicting the disease before it manifests to avoid severe tissue damage (Rose 2015; Miller 2022).

In the future, it is likely that the diagnostic sector will continue to grow and evolve, as scientists and medical professionals work to develop new and more effective diagnostic tools. This may include the use of genetic testing, which can help to identify people who are at high risk of developing autoimmune diseases (Miller, 2022). Additionally, research is focused on developing new drugs and therapies that can help to treat autoimmune diseases more effectively.

Autoimmune diseases are a significant and growing concern in modern medicine. With millions of people affected worldwide and healthcare costs reaching into the billions of dollars, more research is needed to understand the underlying causes of autoimmunity and develop new and effective treatments. The future of the diagnostic sector looks promising with new technologies and diagnostic tools being developed to help identify autoimmune diseases early, and hopefully improve the quality of life for those affected.

Global immune disorders spending from 2011 - 2023
(in billion U.S. dollars).

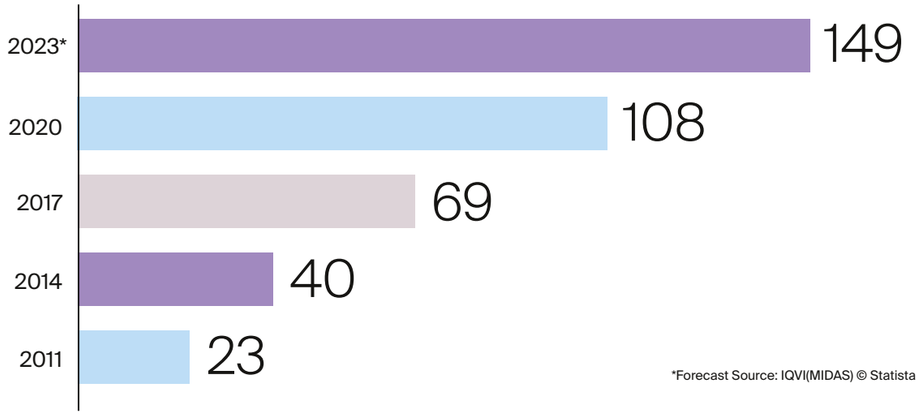


Figure: Global immune disorders spending from 2011 - 2023 in billion U.S. dollars. Healthcare system spendings due to autoimmunity disorders in the past years and in the coming year increased and will increase constantly.

Infobox

- BBI Freiburg, founded in 1999
- Supplier of diagnostic components to the diagnostic industry, similar to BBI
- Specialized on (mostly) recombinant antigens for serological disease state antibodies
- Autoimmunity, allergy, infectious disease
- Quality management according EN ISO 13485

Systemic vs. organ-specific autoimmune diseases

Systemic autoimmune diseases

=> autoantibodies are directed against structures found in almost **any cell** type of the body. Typical systemic autoimmune diseases are:

- Rheumatoid arthritis
- Systemic Lupus Erythematosus
- Scleroderma
- Dermatomyositis

Organ-specific autoimmune diseases

=> autoantibodies are directed against structures of a **specific cell** type. Typical organ-specific autoimmune diseases are:

- Celiac Disease
- Autoimmune thyroid diseases
- Autoimmune liver diseases
- Diabetes
- Crohn‘s Disease

ANA testing algorithm

With the clinical suspicion of Systemic Lupus Erythematosus (SLE) an IFA ANA screen on HEp-2 cells can be ordered by a physician to rule out or rule in an autoimmune disease.

The IFA staining pattern as well as the titer can give a first indication of which type of autoimmune disease to suspect. Subsequent serological assays can then help to further narrow down the exact nature of the disease or rule out others.

ANA testing algorithm – suspected disease

SLE	Systemic Lupus Erythematosus
Sis	Sjögren's Syndrome
SSc	Systemic Sclerosis
MCTD	Mixed Connective Tissue Disease
IIM	Idiopathic Inflammatory Myopathies
RA	Rheumatoid arthritis
APS	Antiphospholipid Syndrome

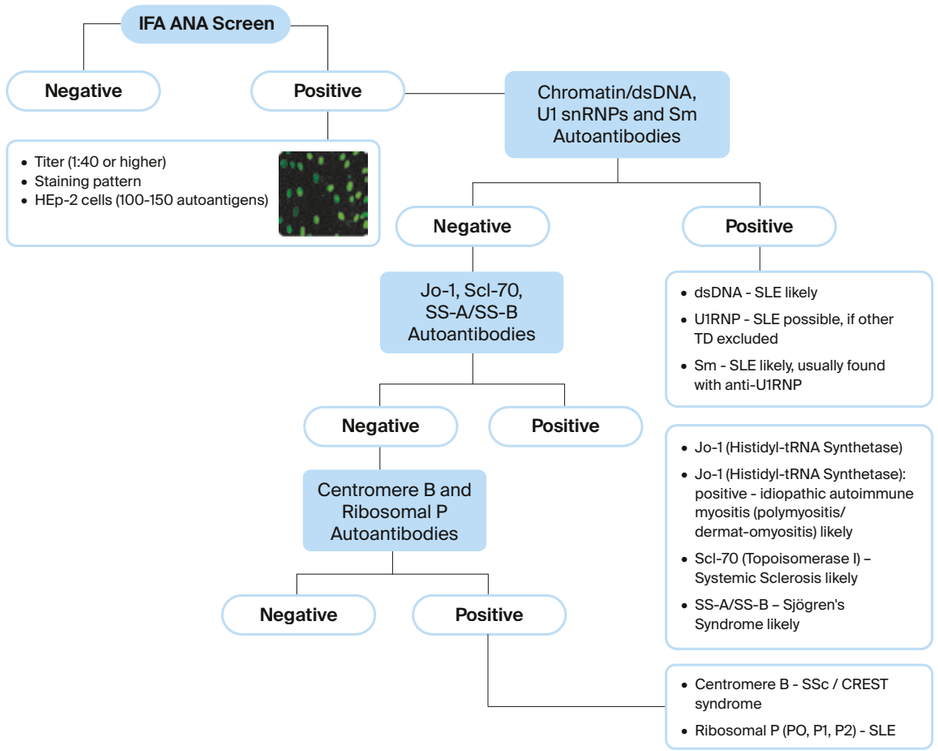


Figure: ANA Testing algorithm.
(Modified after: Kumar et al., diagnostic Pathology (2009); Conrad et al., Autoantibodies in the Systemic Autoimmune diseases (3rd edition; 2015))
This graphic is meant for informational purpose only and is not intended to be used for medical advice.

Autoantigen considerations

Indentity

- Extreme range in biochemical nature of autoantigens
  - Phospholipids, Glycolipids
  - Proteins, Protein Complexes
  - Nucleic Acids
  - Nucleic Acid+Protein Complexes
- In some cases even specific post-translational modifications (PTM) on proteins can define the autoantigen

Source

- Occasional use of human blood or operation material (e.g. thyroid, amputations) for autoantigen preparation
- Historic use of animal tissues when human organ material cannot be ethically sourced (typically bovine or porcine tissue)
- Recombinant expression for targets underrepresented in tissue
- Recombinant expression generally applied for a return to antigen products with authenic human sequence

Reactivity

- Linear epitopes = short sections of unfolded protein
  - Few restrictions on antigen
- Conformational epitopes = autoantigen must be natively folded for proper immunoreactivity
  - Demanding: gentle purification and handling required
- Requirement for PTM can be challenging

Purity

- Very strict requirements:
  - Danger of false positive signals from contaminating antigens
  - Contaminants immediately complicate immobilisation efficiency on immunoassay matrices
- Improved antigen preparation techniques provide highly pure antigens

Supply and properties

- Consistency in access, supply and autoantigen product properties
- Historically, this has not been available to the industry: the very impure historic preparations were produced by non-stable and non-scalable processes base on patient serum
- Today scalability and reproducibility can be assured by more modern techniques such as recombinant expression, immunoaffinity purification etc.

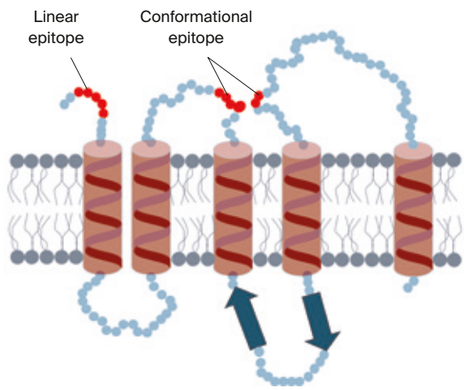
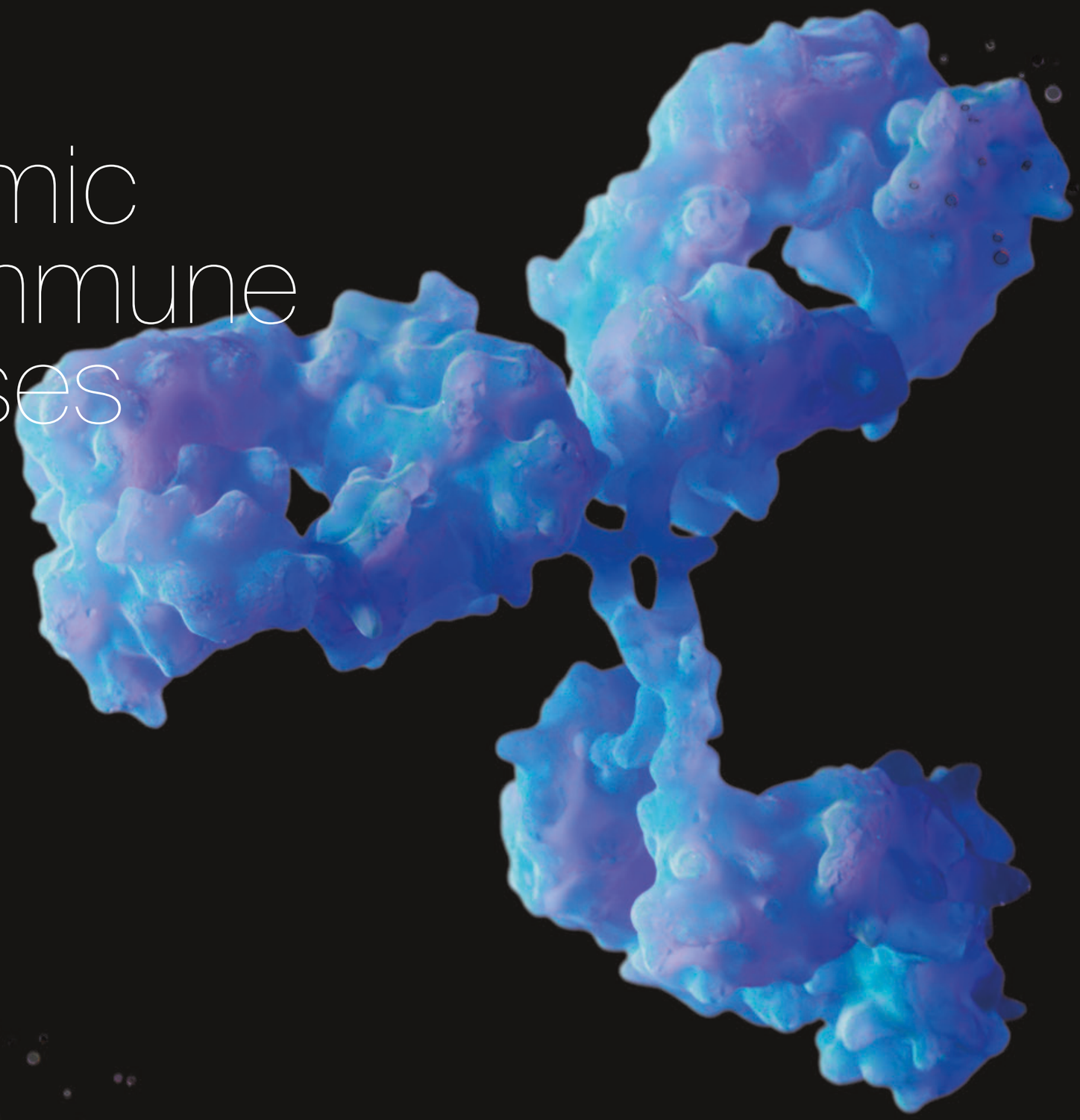


Figure: Linear and conformational epitopes in a transmembrane protein. While the linear epitope will potentially be detectable by an antibody in a denatured protein still, detection of the conformational epitope could be impaired when the protein is improperly folded.

Systemic autoimmune diseases



Systemic Lupus Erythematosus (SLE)

Antigens associated with Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus (SLE) is a chronic, inflammatory autoimmune disease of the connective tissue, which can affect virtually any part of the human body. In line with the complexity of SLE specific symptoms, more than 100 autoantibodies have been associated with this disease.

Although several autoantibodies are considered to be specific for SLE, autoantibodies are known that are also detected in the serum of patients diagnosed with other diseases. Intriguingly, several studies found at least one SLE associated autoantibody in up to 88% of the patients and up to 9.4 years prior to the onset of diagnostic symptoms. In summary, this highlights the necessity of parameters suitable for the high throughput screening of sera from presumed SLE patients.

BBI produces several autoantigens against which SLE associated autoantibodies, including those preceding symptoms, have been identified.

These antigens include Ro/SS-A (60 kDa), U1-snRNP 68/70 kDa and double stranded DNA (dsDNA), which are produced in the E. coli system. Especially autoantibodies against dsDNA are an important diagnostic parameter, which have a prevalence of up to 55% before and 70- 98% at the time of diagnosis. Additional antigens are produced in the baculovirus/insect cell expression system. These include but are not limited to Ro/SS-A (52 kDa), La/SS-B, U1-snRNP A, Sm proteins like U-snRNP B/B', ribosomal phosphoproteins P0-P2, proliferating cell nuclear antigen (PCNA) and the heterodimeric Ku (p70/p80) protein.

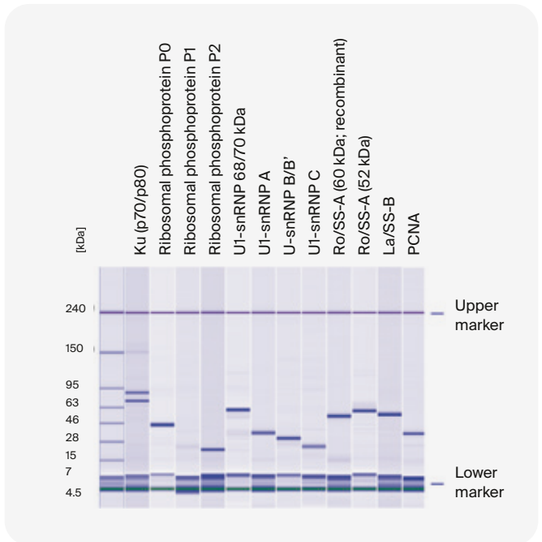


Figure: Electrophoretic analyses of recombinant autoantigens. The loading buffer added to the individual protein preparations contained an upper and lower marker. The molecular weight of the protein standards included in the size ladder are indicated on the left: Electrophoretic analyses of recombinant autoantigens.The loading buffer added to the individual protein preparations contained an upper and lower marker. The molecular weight of the protein standards included in the size ladder are indicated on the left.

References:
Arbuckle et al. (2003) N Engl J Med. 349: 1526-1533
Cozzani et al. (2014) Autoimmune Dis. 2014: 321359
Eriksson et al. (2011) Arthritis Res Ther. 13: R30
Heinlen et al. (2010) PloS One. 10: e9599
Heinlen et al. (2010) J Mol Med (Berl). 88: 719-727
Sherer et al. (2004) Semin Arthritis Rheum. 34: 501-537

Ordering information

Product description	Codes	Size
U1-snRNP 68/70 kDa	13000 13001	50 µg 1.0 mg
U1-snRNP A	13100 13101	50 µg 1.0 mg
U1-snRNP C	13200 13201	50 µg 1.0 mg
U-snRNP B/B'	13300 13301	50 µg 1.0 mg
dsDNA (plasmid)	12300 12301	50 µg 1.0 mg
Ribosomal Phosphoprotein P0	14100 14101	50 µg 1.0 mg
Ribosomal Phosphoprotein P1	14200 14201	50 µg 1.0 mg
Ribosomal Phosphoprotein P2	14300 14301	50 µg 1.0 mg
Proliferating Cell Nuclear Antigen (PCNA)	15400 15401	50 µg 1.0 mg
Ku (p70/p80)	17300 17301	50 µg 1.0 mg
Ro/SS-A (60 kDa; recombinant)	17400 17401	50 µg 1.0 mg
Ro/SS-A (60 kDa; non recombinant; bovine)	15500 15501	50 µg 1.0 mg
Ro/SS-A (52 kDa)	12700 12701	50 µg 1.0 mg
La/SS-B	12800 12801	50 µg 1.0 mg

In some countries the use of certain antigens in diagnostic tests may be protected by patents. BBI is not responsible for the determination of these issues and suggests clarification prior to use.

Systemic Lupus Erythematosus (SLE)

U1-snRNP A, U1-snRNP C, and U1-snRNP 68/70 kDa Antigens

Small nuclear ribonucleoprotein complexes (snRNP) are essential for the splicing of mRNA precursor molecules. U1-snRNP is the most abundant RNP particle in the nucleus and consists of one small uridylate-rich RNA (U1-RNA) complexed with several proteins.

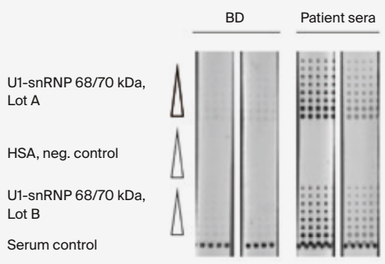


Figure 1: Immunodot analyses of increasing amounts of two different lots of recombinant U1-snRNP 68/70 kDa using sera from blood donors (BD) and patients with mixed connective tissue disease. To ensure specific antibody binding, human serum albumin (HSA) as a negative control and a serum control were also spotted on the nitrocellulose membrane.

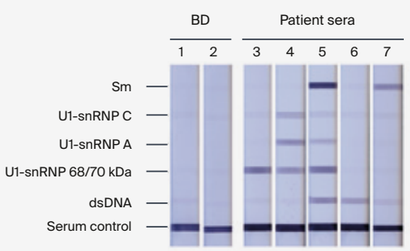


Figure 2: Analyses of sera from blood donors (BD) and patients with mixed connective tissue disease (3-4) and SLE (5-7) for the presence of autoantibodies using line assays. Besides recombinant U1-snRNP proteins native Sm purified from bovine tissue (Sm) and double-stranded DNA (dsDNA) were included in the assay to better visualize differences in autoantibody patterns.

The three proteins 68/70 kDa, A, and C are unique to the U1-snRNP particle, whereas seven so-called Sm proteins (B/B', D1, D2, D3, E, F, G) form a core subparticle that is common to all U-snRNP complexes.

Both the U1-specific proteins and the Sm core particle are targets of autoantibodies, which classically have been called the RNP and RNP/Sm antigens, respectively. The nomenclature of the U1-snRNP 68/70 kDa protein refers to the fact that different splice variants of this protein are found in human cells. A clear diagnostic distinction of the specificities of these autoantibodies has been complicated by the biochemical difficulties of producing homogeneous subparticle fractions from native sources. The use of single recombinant proteins as antigenic targets guarantees a much higher sensitivity and specificity in immunodiagnostic assays. Recombinant RNP and RNP/Sm antigens do not only allow the discrimination between these autoantibodies, but also the detection of autoantibodies that might be missed in diagnostic assays using the RNP/Sm complex due to sterical hindrances making epitopes inaccessible.

Autoantibodies to U1-snRNP specific proteins are present in 95% of patients with mixed connective tissue disease (MCTD) and are considered as a serological hallmark. Especially antibodies against the U1-snRNP 68/70 kDa protein are known to have a high clinical significance in MCTD patients. However, these autoantibodies are also detected in 30% of patients with Systemic Lupus Erythematosus (SLE). On the contrary, RNP/Sm autoantibodies appear to be restricted to SLE patients, although at a low sensitivity, and are therefore also considered a serological hallmark, which further highlights the importance of recombinant RNP and RNP/Sm antigens in the diagnosis of MCTD and SLE.

U1-snRNP A and U1-snRNP C are produced in the baculovirus/insect cell expression system. U1-snRNP 68/70 kDa is produced in E. coli.

References:
Cozzani et al. (2014) Autoimmune Dis. 321359
Sharp et al. (1972) Am J Med. 52:148-159
Tani et al. (2014) J Autoimmune. 48-49:46-49

Systemic Lupus Erythematosus (SLE)

Histone, Nucleosome and dsDNA Antigens

Histones, the major components of nucleosomes, are responsible for establishing chromatin structure in the nucleus of eukaryotic cells.

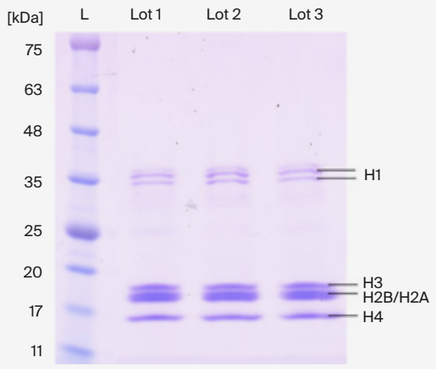


Figure 2: SDS-PAGE of three independent lots of non-recombinant DIARECT histones (including histone H1, H3, H2A, H2B and H4). The molecular weight of protein standards included in the size ladder (L) are indicated on the left.

The histone octamer of each nucleosome core particle contains a (H3-H4)2 tetramer, organizing the central part of the DNA, and two flanking H2A-H2B dimers. Histone H1, the linker histone, is located between each nucleosome (MacAlpine and Almouzni 2013).

Histones H3 and H4 have long tails protruding from the nucleosome, which can be covalently modified (methylation, acetylation, phosphorylation and ubiquitination) at several residues. The combination of those modifications and those of core histones H2A and H2B constitute the so-called histone code (Strahl and Allis 2000; Jenuwein et al. 2001).

Dynamics of chromatin structure depend on posttranslational modification of histones and appearance of various his-tone variants. This is important for different molecular mechanisms including DNA repair, transcription, replication, recombination, control of gene expression and epigenetic responses to external signaling (Maeshima et al. 2014).

The chronic autoimmune disease systemic lupus erythe-matosus (SLE) can involve several organs and systems within the human body and is characterized by production of various autoantibodies (Cozzani et al. 2014).

Anti-nucleosome antibodies (ANuAs) are directed against histone epitopes, dsDNA and conformational epitopes created by the interaction between dsDNA and core histones. ANuAs have been shown to be a good diagnostic marker for systemic lupus erythematosus (SLE). Antibodies to histones (AHAs) are even more common autoantibodies seen in patients with SLE (Bizzaro et al. 2012) and are directed towards epitopes of histone complexes or individual histones (Burlingame et al. 1994; Santiago et al. 2007).

Complementing the dsDNA plasmid produced in E. coli and the nucleosome antigen from calf thymus, BBI offers isolated histone antigen also purified from calf thymus.

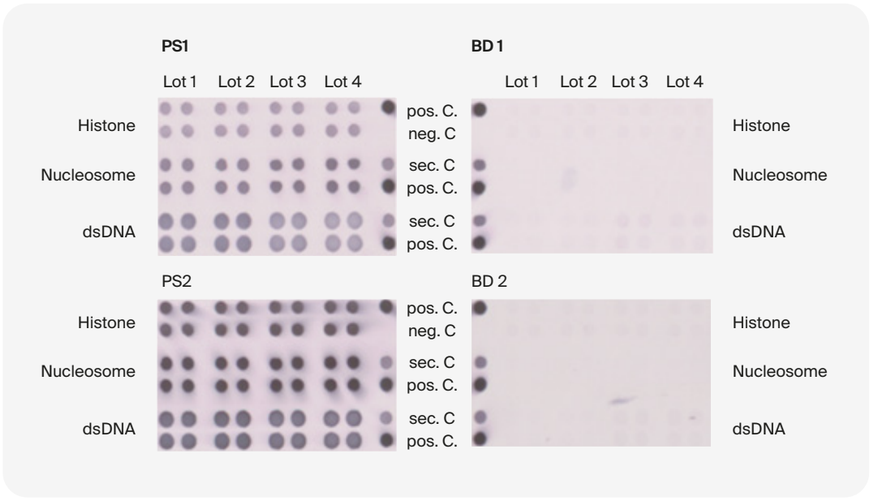


Figure: Immunodot analyses of histone, nucleosome and dsDNA antigens with sera from SLE patients (PS1-2) and blood donors (BD1-2). The presence of histone, nucleosome, and dsDNA antibodies was determined by spotting quadruplicates of recombinant dsDNA antigen produced in E. coli and non-recombinant histone and nucleosome antigens purified from calf thymus. On one side of each array positive (pos. C.), negative (neg. C.) and secondary antibody incubation controls (sec. C.) were printed.

Ordering information

Product description	Codes	Size
dsDNA (plasmid)	12300 12301	50 µg 1.0 mg
Histone (non recombinant; bovine)	31100 31101	50 µg 1.0 mg
Nucleosome (non recombinant; bovine)	31000 31001	50 µg 1.0 mg

References:
Bizzaro et al. (2012) Autoimmun Rev. 12 (2): 97-106
Burlingame et al. (1994) J Clin Invest. 94 (1): 184-192
Cozzani et al. (2014) Autoimmune Dis. 2014: 321359
Jenuwein et al. (2001) Science. 293 (5532): 1074-1080
MacAlpine and Almouzni (2013) Cold Spring Harb Perspect Biol. 5 (8): a010207
Maeshima et al. (2014) Chromosoma. 123 (3): 225-237
Santiago et al. (2007) J Rheumatol. 34 (7): 1528-1534
Strahl and Allis (2000) Nature. 403 (6765): 41-45

Systemic Lupus Erythematosus (SLE)

Ribosomal Phosphoproteins P0, P1 and P2

The eukaryotic ribosome is composed of a 40S and 60S subunit. While the 40S subunit comprises one ribosomal RNA and 33 different basic proteins, the 60S subunit comprises three ribosomal RNAs and 46 different basic proteins and, in addition, three phosphorylated and acidic proteins.

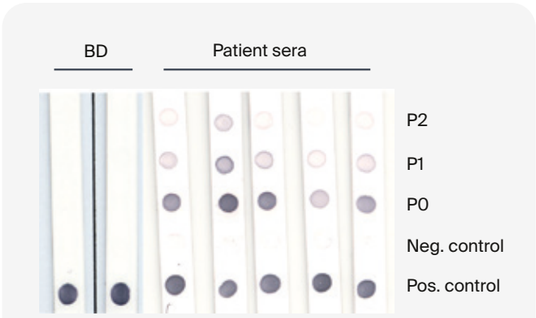


Figure: Immunodot analyses of sera from blood donors (BD) and patients with presumed systemic lupus erythematosus for autoantibodies against ribosomal phosphoproteins P0, P1 and P2.

These so-called ribosomal phosphoproteins (P0, P1, P2) form a pentameric complex consisting of one P0, two P1 and two P2 proteins (Barkhudarova et al. 2011; Elkon et al. 1986; Kiss et al. 2007).

Systemic Lupus Erythematosus (SLE) is a debilitating and chronic life threatening tissue disease that can virtually affect any organ. Early diagnosis is essential to alleviate the progression of SLE. Although more than 100 autoantibodies have been associated with SLE, complicating its diagnosis, several autoantibodies have been reported to be detectable years before symptoms (Arbuckle et al. 2003; Cozzani et al. 2014; Sherer et al. 2004).

Autoantibodies against the ribosomal phosphoproteins are, on average, detected in approximately 15–30% of all SLE patients. However, a prevalence of up to 40% has been found for patients from Asia. The importance of autoan-tibodies against ribosomal phosphoproteins is further high-lighted by studies reporting that these autoantibodies can be detected up to 1.7 years prior to the diagnosis of SLE (Arbuckle et al. 2003; Heinlen et al. 2010).

Although autoantibodies against double-stranded DNA (dsDNA) and the spliceosomal Sm proteins are considered the hallmarks of SLE serology, SLE patients who are serologically negative for these autoantibodies are known. Intriguingly, 10–28% of these patients were positive for autoantibodies against ribosomal phosphoproteins underlining their role in SLE serology (Li et al. 2013).

References:
Arbuckle et al. (2003) N Engl J Med. 349 (16): 1526-1533
Barkhudarova et al. (2011) Arthritis Res Ther. 13 (1): R20
Cozzani et al. (2014) Autoimmune Dis. 2014: 321359
Elkon et al. (1986) PNAS. 83 (19): 7419-7423
Heinlen et al. (2010) J Mol Med (Berl). 88 (7): 719-727
Kiss et al. (2011) Clin Rev Allergy Immunol. 32 (1): 37-46
Li et al. (2013) J Clin Lab Anal. 27 (2): 87-95
Mahler et al. (2003) J Mol Med. 81 (3): 194-204
Mahler et al. (2008) Arthritis Res Ther. 10 (6): R131
Sherer et al. (2004) Semin Arthritis Rheum. 34 (2): 501-537

Historically, autoantibodies against the ribosomal phosphoproteins were detected by their cytoplasmic pattern in indirect immunofluorescence (IIF) (Mahler et al. 2008). Due to false-negative results, other immunoassays using purified native ribosomal phosphoprotein complex or recombinant P0 - P2 have been established.

Ordering information

Product description	Codes	Size
Ribosomal Phosphoprotein P0	14100 14101	50 µg 1.0 mg
Ribosomal Phosphoprotein P1	14200 14201	50 µg 1.0 mg
Ribosomal Phosphoprotein P2	14300 14301	50 µg 1.0 mg

Since all three ribosomal phosphoproteins share a major immunodominant C-terminal domain, the so-called C22 peptide, commercial assays using this peptide as antigen have also been established (Elkon et al. 1986). However, autoantibodies against other epitopes, e.g., residues 99–113 (Epitope 3) of P0 or conformational epitopes, have been identified (Mahler et al. 2003). In addition, Heinlen et al. (2010) reported that autoantibodies against Epitope 3 were detectable prior to autoantibodies against the C-22 peptide.

When comparing the sensitivity of ELISA for the detection of autoantibodies against ribosomal phosphoproteins, Barkhudarova et al. (2011) and Li et al. (2013) reported that, at a specificity cut off of 99%, individual recombinant ribosomal phosphoproteins outcompete the native ribosomal phosphoprotein complex as coating antigens. As suggested by the authors, this might be due to the inaccessibility of certain epitopes in the complex.

BBI produces full length ribosomal phosphoproteins P0, P1 and P2 in the baculovirus/insect cell expression system.

SSC/Scleroderma

Centromere Proteins CENP-B and CENP-A

The centromere is a defined DNA region of a chromosome where the sister chromatids are connected, and the attachment site of the kinetochore. The latter connects the chromosomes to the microtubules of the spindle apparatus, which segregates the sister chromatids during mitosis.

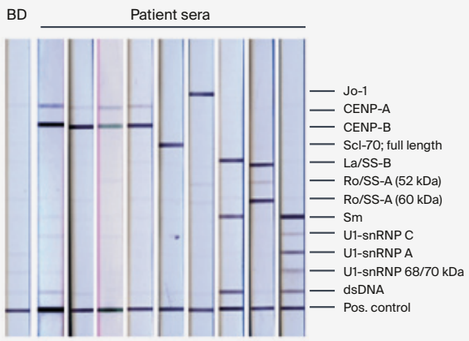


Figure: Analyses of sera from a blood donor (BD) and patients with presumed systemic sclerosis/scleroderma for the presence of autoantibodies using line assays. Besides recombinant CENP-A and CENP-B a select panel of other recombinant antigens including double-stranded DNA (dsDNA) were included in the assay.

At least nine different proteins associated with the cen-tromere, the so-called centromere proteins (CENP), have been identified and termed CENP-A through I. CENP-A and CENP-B are among the best studied CENP proteins. CENP-A is a histone H3 variant that is specifically found in nucleosomes at the centromere. CENP-B is a DNA-binding protein that recognizes the so-called CENP-B boxes located in the centromeric DNA, and is involved in the assembly of centromeric structures including the kinetochore.

Anti-centromere autoantibodies (ACA) have been first described by Moroi et al. in 1980 by analyzing sera from systemic sclerosis (SSc)/scleroderma patients using indirect immunofluorescence (IIF). While in the interphase nucleus a punctuated but dispersed signal pattern was observed, this pattern became restricted to the centromere/kinetochore during mitosis. Depending on the study, ACA are reported to have an overall prevalence of 20-40% among SSc patients.

SSc/scleroderma is a generalized term for a systemic connective tissue disorder affecting skin and internal organs, which is characterized by fibrotic arteriosclerosis of peripheral and visceral arteries. A more limited form of SSc/slceroderma, termed CREST syndrome, has been described.

References:
Cheeseman (2014) Cold Spring Harb Perspect Biol. 6 (7): a015826
Gonzalez-Buitrago et al. (2003) Clin Chim Acta. 328 (1-2): 135-138
Hamaguchi (2010) J Dermatol. 37 (1): 42-53
Hudson et al. (2012) J Rheumatol. 39 (4): 787-794
Mahler et al. (2011) Clin Chim Acta. 412 (21-22): 1937-1943
Moroi et al. (1980) PNAS. 77 (3): 1627-1631
Nakamura et al. (2010) BMC Musculoskelet Disord. 11: 140
Russo et al. (2000) J Rheumatol. 27 (1): 142-148
Varga et al. (2007) J Clin Invest. 117 (3): 557-567

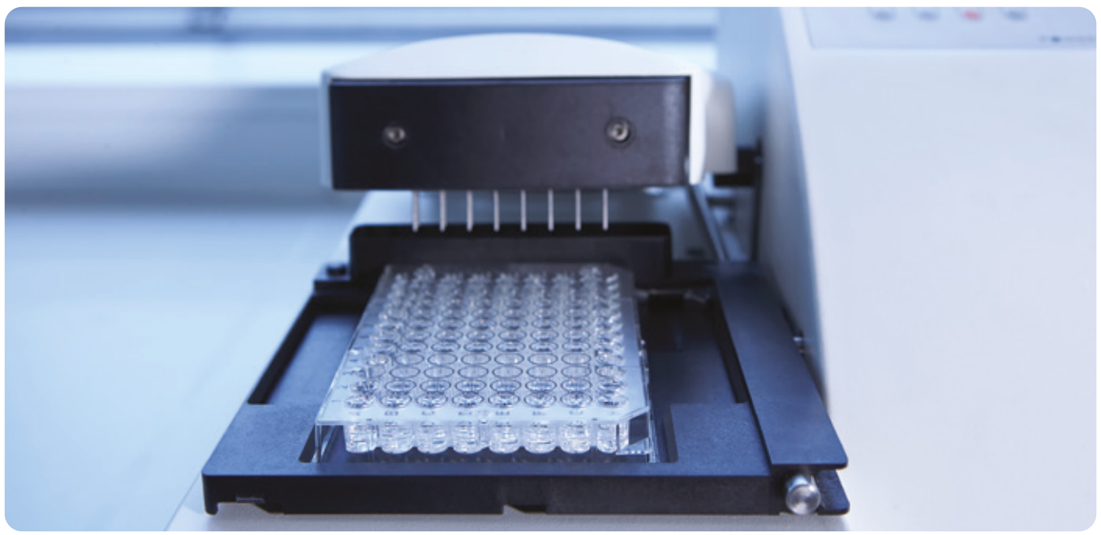
Studies following the initial description of ACA identified CENP-A and CENP-B to represent major ACA antigens. In general, ACA appear to correlate with a more limited form of SSc/Scleroderma. This is supported by the finding that CENP-B autoantibodies exert a prevalence of up to 80% in patients diagnosed with CREST syndrome.

Ordering information

Product description	Codes	Size
Ribosomal Phosphoprotein P0	**14100** **14101**	**50 µg** **1.0 mg**
Ribosomal Phosphoprotein P1	**14200** **14201**	**50 µg** **1.0 mg**
Ribosomal Phosphoprotein P2	**14300** **14301**	**50 µg** **1.0 mg**

In CENP-B negative patients, CENP-A autoantibodies represent an important marker. Within the last couple of years, the availability of recombinant CENP-A and CENP-B enabled the high-throughput screening of patient sera for the respective autoantibodies using solid-phase based methods, e.g. ELISA. Recently, several studies reported that ELISA tests using recombinant CENP-A and CENP-B are similar or even superior to IIF in detecting CENP-A and CENP-B autoantibodies. ribosomal phosphoproteins P0, P1 and P2 in the baculovirus/insect cell expression system.

CENP-B and CENP-A antigens from BBI are produced in the baculovirus/insect cell expression system.



Idiopathic Inflammatory Myopathies (IIMS)

Antigens in Idiopathic Inflammatory Myopathies

Idiopathic Inflammatory Myopathies (IIMs) are characterized by the presence of inflammatory infiltrates within skeletal muscle and are defined by a variety of syndromes. The most prevalent subtypes include adult Polymyositis (PM) and Dermatomyositis (DM), along with Inclusion Body Myositis (IBM) and myositis in overlap with another autoimmune connective tissue disease (Overlap Syndrome).

(Betteridge et al. 2011; Bohan et al. 1975a/b)

DM and PM are diseases with different pathophysiological mechanisms. DM has been found to be humorally elicited while PM seems to be caused by a T-cell mediated mechanism (Gherardi 2011). The main difference from a clinical perspective is that the skin is involved in DM but not in PM while symptoms such as muscle inflammation are characteristic for both diseases (Mammen 2010).

To date, a variety of autoantibodies have been identified to be involved in the onset of IIMs and these can function as biomarkers for further demarcating the subtypes of the disease (Betteridge and McHugh 2016; Targoff et al. 1992). These autoantibodies can be further categorized into myositis specific autoantibodies (MSAs) and myositis associated autoantibodies (MAAs) with the earlier being a mainly exclusive PM/DM marker and the later also occurring in other connective tissue diseases (Betteridge and McHugh 2016; Love et al. 1991). Many MSAs are also associated with a unique clinical subset of PM/DM, making them useful in predicting and monitoring certain clinical manifestations (Satoh et al. 2017) (Figure 1).

Autoantibodies targeting eight of the 20 aminoacyl-tRNA synthetases (anti-ARS antibodies) have been identified so far, being found in up to 30% of sera from patients with myositis (Satoh et al. 2017). They are highly specific for this disorder and strongly associated with complicating lung disease (ILD) (Betteridge et al. 2011; Hirakata et al. 1999). The most prevalent one identified in 20 % of IIM patients is Jo-1; antibodies against other ARS can collectively be found in another 20% of patients (Gunawardena et al. 2009; Satoh et al. 2017).

Although the anti-synthetase syndrome (ASS) comprises all eight synthetases, the symptoms associated with each autoantibody are slightly different. While patients with antibodies against Jo-1 show the classic PM symptoms, other patients with autoantibodies against OJ, KS or PL-12 can also exhibit DM-like skin lesions and they are very likely to develop ILD. Patients with autoantibodies against PL-7 show a milder muscle weakness compared to the one observed in patients with anti-Jo-1 antibodies (Betteridge et al. 2011).

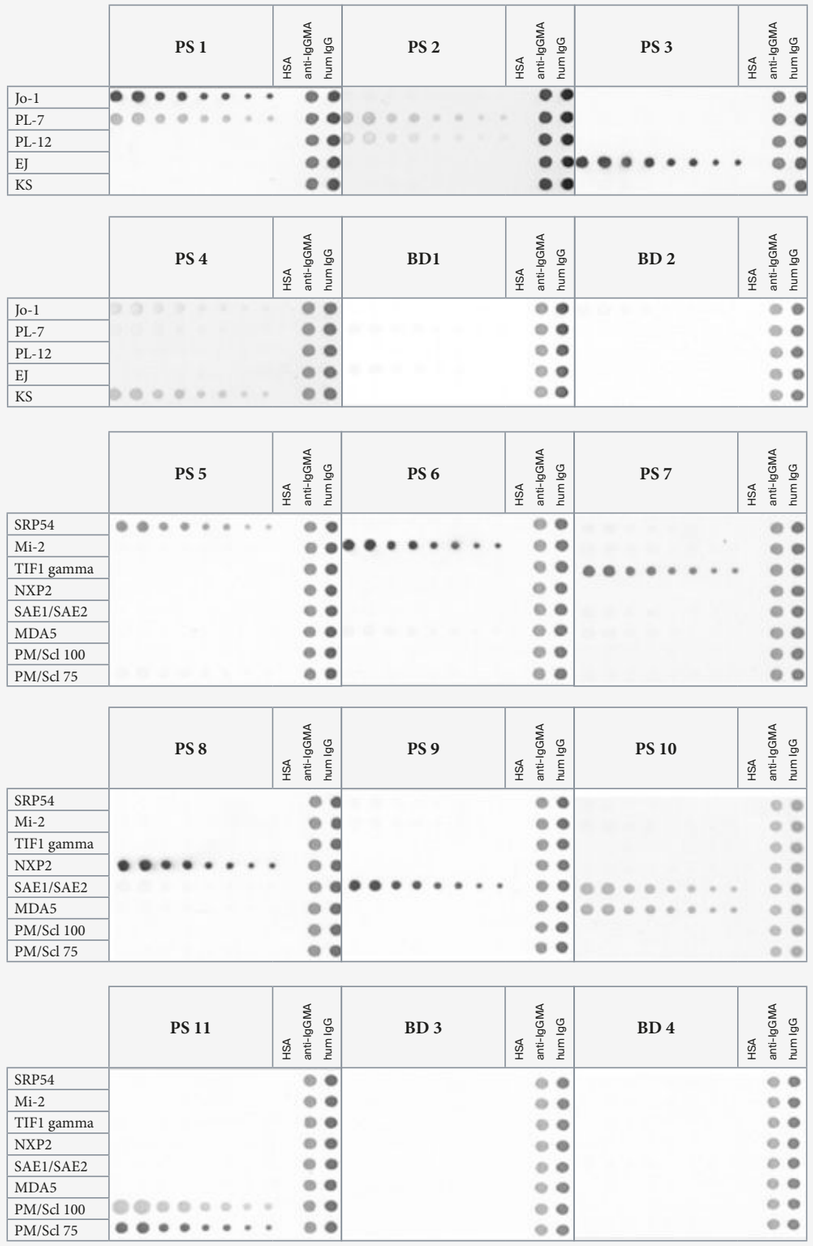


Figure: Immunodot analyses of negative (BD 1 - 4) and positive samples (PS 1 - PS 11) for myositis. The presence of myositis autoantibodies was determined by spotting decreasing amounts of selected recombinant myositis antigens produced in the baculovirus / insect cell expression system. Proteins and controls (HSA, anti-IgGMA and hum IgG) were printed on nitrocellulose membrane as indicated.

Idiopathic Inflammatory Myopathies (IIMS)

Antigens in Idiopathic Inflammatory Myopathies

Anti-Signal Recognition Particle (SRP) antibodies are characteristic for PM and are mainly associated with a syndrome of a necrotizing myopathy with cardiac involvement, severe prognosis and poor response to therapy.

(Betteridge et al. 2011; Miller et al. 2002; Reeves et al. 1986)

Until now, they have not been described in patients with DM involvement or overlap syndrome (Targoff et al. 1990).

Anti-Mi-2 autoantibodies are considered specific serological markers of DM. Detected in about 20% of myositis sera, they are proven markers for acute onset, good prognosis and good response to therapy (Ghirardello et al. 2014; Satoh et al. 2017; Targoff and Reichlin 1985).

More recent publications described a number of novel autoantibodies especially in DM patients (Satoh et al. 2017). Autoantibodies to anti-p155/140 (TIF1 gamma) are found in up to 20% of patients with DM. A strong link with cancer associated myositis was shown in anti-TIF1 gamma positive patient cohorts (Targoff et al. 2006; Fujimoto et al. 2012).

Autoantibodies to the cytoplasmic melanoma differentiation antigen 5 (MDA5) have been mainly reported in Asian patients suffering from DM. In addition, the occurrence of this novel autoantibody was shown to be associated with interstitial lung disease (Sato et al. 2005; Sato et al. 2013).

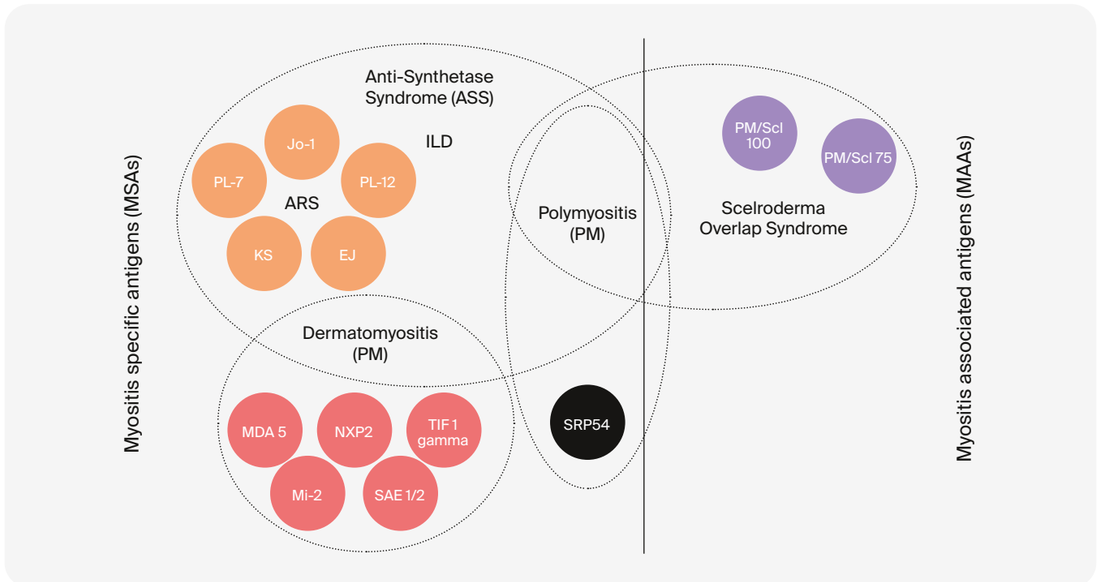


Figure: Categorization of BBI's antigens in idiopathic inflammatory myopathies (IIM).

Autoantibodies directed against nuclear matrix protein 2 (NXP2), a 140kDa protein situated in the nuclear matrix, have been reported in about 25% of DM and very rarely in PM patients. NXP2 plays a role in the regulation of p53-induced apoptosis and autoantibodies against this protein had been associated with a higher risk for malignancies.

(Ceribelli et al. 2012; Ghirardello et al. 2014; Ichimura et al. 2012)

Ordering information

Product description	Codes	Size
Histidyl-tRNA Synthetase (Jo-1)	12900 12901	50 µg 1.0 mg
Threonyl-tRNA Synthetase (PL-7)	15600 15601	50 µg 1.0 mg
Alanyl-tRNA Synthetase (PL-12)	15700 15701	50 µg 1.0 mg
Glycyl-tRNA Synthetase (EJ)	11100 11101	50 µg 1.0 mg
Asparaginyl-tRNA Synthetase (KS)	30100 30101	50 µg 1.0 mg
SRP54	18400 18401	50 µg 1.0 mg
Mi-2	18100 18101	50 µg 1.0 mg
TIF1 gamma	11000 11001	50 µg 1.0 mg
SAE1/SAE2	31600 31601	50 µg 1.0 mg
NXP2	31700 31701	50 µg 1.0 mg
MDA5	30000 30001	50 µg 1.0 mg
PM/Scl 100	16000 16001	50 µg 1.0 mg
PM/Scl 75	17000 17001	50 µg 1.0 mg
La/SS-B	12800 12801	50 µg 1.0 mg

In 2009, Betteridge et al. were the first to associate antibodies that target the small ubiquitin-like modifier activating enzyme subunits 1 and 2 (SAE1/SAE2) with DM. Three years later, anti-SAE1/SAE2 antibodies had been confirmed as a marker for DM with mainly skin and muscle manifestations and the absence of other symptoms such as interstitial lung disease and arthritis. (Tarricone et al. 2012).

Autoantibodies against PM/Scl (mainly PM/Scl 100) occur in patients suffering from polymyositis, scleroderma or overlap syndrome. They are therefore classified as MAAs meaning that they cannot be specifically correlated with one specific clinical picture but two or more (Betteridge et al. 2011; Koenig et al. 2007). The presence of PM/Scl 100 antibodies had originally been reported to be a "good" prognostic sign in overlap syndrome, unlike the poor prognosis seen when other myositis and systemic sclerosis specific antibodies are present. However, this view was partially revised by Marie et al. in 2010 where 70% of patients positive for PM/Scl improved but 20% had a worsened clinical status after long-term observation.

BBI offers the most complete panel of antigens for IIM characterization. All our recombinant autoantigens are produced in the baculovirus / insect cell expression system.

References:
Betteridge et al. (2007) Arthritis Rheum. 56 (9): 3132–3137
Betteridge et al. (2009) Ann Rheum Dis. 68(10):1621-1625
Betteridge et al. (2011) Arthritis Res Ther. 13 (2): 209
Betteridge and McHugh (2016) J Intern Med. 280 (1): 8–23
Bohan and Peter (1975a) N Engl J Med. 292 (7): 344–347
Bohan and Peter (1975b) N Engl J Med. 292 (8) 403–407
Ceribelli et al. (2012) Arthritis Res Ther. 14 (2): R97
Fujimoto et al. (2012) Arthritis Rheum. 64 (2) 513–522
Gherardi (2011) Presse Med. 40 (4 Pt 2): e209-218
Ghirardello et al. (2014) Auto Immun Highlights. 5 (3): 69–75
Gunawardena et al. (2009) Rheumatology. 48 (6): 607–612
Hirakata et al. (1999) J Immunol. 162 (4): 2315–2320
Ichimura et al. (2012) Ann Rheum Dis. 71 (5): 710–713
Koenig et al. (2007) Arthritis Res Ther. 9 (4): R78
Love et al. (1991) Medicine. 70 (6): 360–374
Mammen (2010) Ann N Y Acad Sci. 1184 (1): 134–153
Marie et al. (2010) Br J Dermatol. 162 (2): 337-344
Miller et al. (2002) J Neurol Neurosurg Psychiatry. 73 (4): 420–428
Reeves et al. (1986) Proc Natl Acad Sci USA. 83 (24): 9507–9511
Sato et al. (2005) Arthritis Rheum. 52 (5): 1571–1576
Sato et al. (2013) Mod Rheumatol. 23 (3): 496–502
Satoh et al. (2017) Clinic Rev Allerg Immunol. 52 (1): 1–19
Targoff (1992) Rheum Dis Clin North Am. 18 (2): 455–482
Targoff (1990) Arthritis Rheum. 33 (9): 1361–1370
Targoff et al. (2006) Arthritis Rheum. 54 (11): 3682–3689
Targoff and Reichlin (1985) Arthritis Rheum. 28 (7): 796–803
Tarricone et al. (2012) J Immunol Methods. 384 (1-2): 128–134

Organ-specific autoimmune diseases

Type 1 diabetes

GAD65 and IA-2 Antigens

Insulin-dependent diabetes mellitus (IDDM) or Type 1 diabetes mellitus (T1DM) is a T-cell mediated autoimmune disorder characterized by destruction of pancreatic beta cells[1]. Mostly starting in childhood, this leads to insulin deficiency and metabolic abnormalities[2].

1. Ziegler et al. 2013

2. Pihoker et al. 2005). Patients require lifelong insulin treatment (Landin-Olsson et al. 1992

In the 1970s it was described that beta cell destruction is associated with the production of autoantibodies to cytoplasmic antigens of islet cells (ICAs) (Bottazzo et al. 1974). Using classic diagnostic ICA tests, polyclonal antibodies are detected in approximately 85% of children with recently diagnosed T1DM (Winter et al. 2002).

In the early 1990s, new antigens were identified including a 37/40 kDa tryptic fragment, which was identified to belong to the putative tyrosine phosphatase insulinoma-associated protein (IA-2), as well as the glutamate decarboxylase (GAD) antigen (Bækkeskov et al. 1990; Passini et al. 1995).

GAD is a pyridoxal phosphate-dependent enzyme that catalyzes the irreversible decarboxylation of glutamate to form gamma-aminobutyrate (GABA). Named according to its respective molecular weight, the pancreatic GAD65 isoform contains an N-terminal membrane-anchoring signal peptide and localizes in the proximity of the Golgi apparatus of islet cells and GABA-containing vesicles (Brilliant et al. 1990; Bu et al. 1992; Solimena et al. 1994). GAD65 autoantibodies appear in 70–80% of sera from recently diagnosed T1DM patients (Hagopian et al. 1993). However, they can also be present in nondiabetic individuals and are thus alone not specific (Christie et al. 1994).

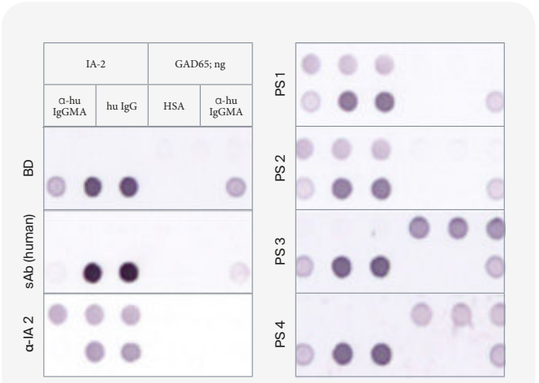


Figure: Immunodot analyses of new generation GAD65; ng and IA-2 in triplicates using a rabbit polyclonal anti-IA-2 antibody (α-IA-2), an anti-human secondary antibody (sAb (human)), sera from T1DM patients (PS1-4) and a blood donor (BD). As positive controls, goat anti-human IgGMA (α-hu IgGMA) and IgG were used. As negative control HSA was spotted on nitrocellulose membrane.

References:
Achenbach et al. (2013) Diabetologia. 56 (7): 1615-1622
Bækkeskov et al. (1990) Nature. 347 (6289): 151-156
Bonifacio et al. (1995) J Immunol. 155 (11): 5419-5426
Bottazzo et al. (1974) Lancet. 2 (7892): 1279-1283
Brilliant et al. (1990) Genomics. 6 (1): 115-122
Bu et al. (1992) PNAS. 89 (6): 2115-2119
Christie et al. (1994) Diabetes. 43 (10): 1254-1259
Hagopian et al. (1993) Diabetes. 42 (4): 631-636
Lampasona et al. (1996) J Immunol. 157 (6): 2707-2711
Landin-Olsson et al. (1992) Diabetologia. 35 (11): 1068-1073
Passini et al. (1995) PNAS. 92 (20): 9412-9416
Pihoker et al. (2005) Diabetes. 54 (2): 52-61
Solimena et al. (1994) J Cell Biol. 126 (2): 331-341
Solimena et al. (1996) EMBO J. 15 (9): 2102-2114
Winter et al. (2002) Clinical Diabetes. 20 (4): 183-191
Winter et al. (2011) Clin Chem. 57 (2): 168-175
Ziegler et al. (2013) JAMA. 309 (23): 2473-2479

IA-2, also called islet cell antigen 512 (ICA 512), is a catalytically inactive protein tyrosine phosphatase (PTP) (Bonifacio et al. 1995). It consists of an N-terminal extracellular signal sequence, a transmembrane domain and a long C-terminal intracellular tail, that harbors the majority of autoantibody epitopes

(Lampasona et al. 1996).

Ordering information

Product description	Codes	Size
Glutamate Decarboxylase 65 kDa (GAD65; ng)	31900 31901	50 µg 1.0 mg
IA-2 (ICA 512)	30500 30501	50 µg 1.0 mg

Similar to GAD it is expressed within secretory granules in neural, neuroendocrine and pancreatic islet cells (Solimena et al. 1996). Compared to anti-GAD antibodies, anti-IA-2 antibodies appear later and are therefore used as predictive value for upcoming T1DM onset in at-risk individuals (Achenbach et al. 2013). IA-2 autoantibodies are detected in 60–80% of sera from individuals with recent onset of the disease (Winter et al. 2011).

BBI 's antigens, GAD65 and IA-2 (ICA 512), are produced in the baculovirus/insect cell expression system.

Autoimmune thyroid disease

Thyroid Peroxidase and Thyroglobulin

Thyroid Peroxidase (TPO) represents one of the main autoantigens in human autoimmune thyroid disease that affects up to 5% of the general population.

Several years ago, it has been shown that TPO and the so-called “microsomal antigen” are identical. TPO is an integral membrane glycoprotein, which is composed of two identical subunits of approximately 100 kDa each, and restricted to the apical plasma membrane of the follicular epithelial cells.

The hemoprotein TPO plays a key role in thyroid hormone biosynthesis by catalyzing both the iodination of tyrosyl residues and the coupling of iodotyrosyl residues in thyroglobulin (TG) to form precursors of the thyroid hormones T4 and T3.

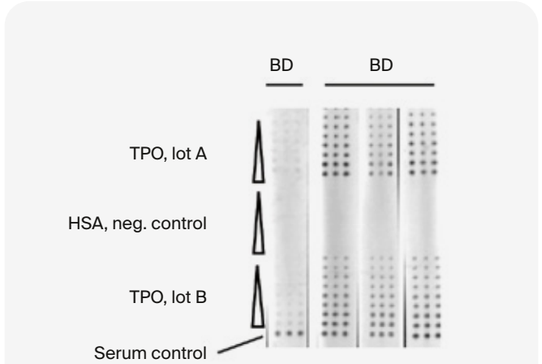


Figure: Immunodot analyses of increasing amounts of two different lots of recombinant TPO using sera from a blood donor (BD) and patients with Hashimoto thyroiditis. To ensure specific antibody binding, human serum albumin (HSA) as negative control and a serum control were also spotted on the nitrocellulose membrane.

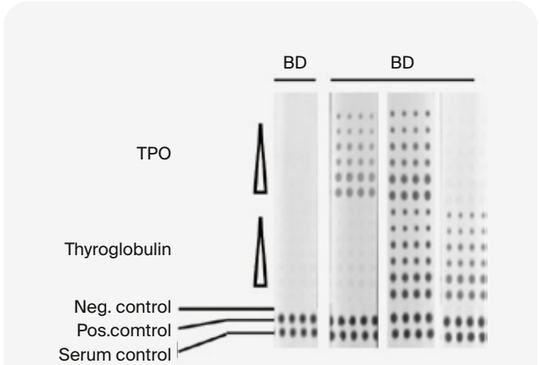


Figure: Immunodot analyses of increasing amounts of recombinant TPO and native thyroglobulin using sera from a blood donor (BD) and patients with Hashimoto thyroiditis.

References:
Caturegli et al. (2014) Autoimmun Rev. 13:391-397
Cooper et al. (2009) J Autoimmun. 33:197-207
Haubruck et al. (1993) Autoimmunity. 15:275-284
Hutfless et al. (2011) J Clin Endocrinol Metab. 96:E1466-E1471
Iddah et al. (2013) ISRN Endocrinol. 2013:509764
Menconi et al. (2014) Autoimmun Rev. 13:398-402
Portmann et al. (1988) J Clin Invest. 81: 1217-1224

TPO autoantibodies, the serological hallmark of human autoimmune thyroid disease, are found with a prevalence of over 90% in patients with Hashimoto thyroiditis, which is now considered one of the most common autoimmune diseases with an annual incidence of up to 1.5 cases per 1000 individuals.

Ordering information

Product description	Codes	Size
Thyroid Peroxidase (TPO)	12100 12101	50 µg 1.0 mg
Thyroglobulin (non recombinant)	12200 12201	50 µg 1.0 mg

In patients diagnosed with the related Grave’s disease, TPO autoantibodies are detected at a lower prevalence ranging from 70 to 90%. A recent study published by Hutfless et al. in 2011 reported that TPO autoantibodies precede by years the diagnostic symptoms associated with the autoimmune thyroid disease phenotype.

BBI’s TPO antigen is produced in the baculovirus/insect cell expression system as a truncated, soluble molecule comprising the extracytoplasmic domain that contains the epitope targeted by TPO autoantibodies. The recombinant production of an engineered TPO antigen did eliminate the purity problems of the classical microsomal antigen preparations from thyroid follicles, which are inevitably contaminated with thyroglobulin.

Thyroglobulin is a large globular, dimeric glycoprotein with a total molecular weight of 660 kDa, and a key precursor in the biosynthesis of the thyroid hormones. It makes up approximately 75% of the thyroid follicles’ protein content and represents another major thyroid autoantigen with up to 80% of Hashimoto thyroiditis and Graves‘ disease patients being serologically positive. Like for TPO autoantibodies, (Hutfless et al. 2011) reported that thyroglobulin autoantibodies precede the development of diagnostic symptoms.

BBI produces native thyroglobulin purified from human thyroid glands.

Autoimmune liver disease

LKM 1, LC 1 and SLA/LP Antigens

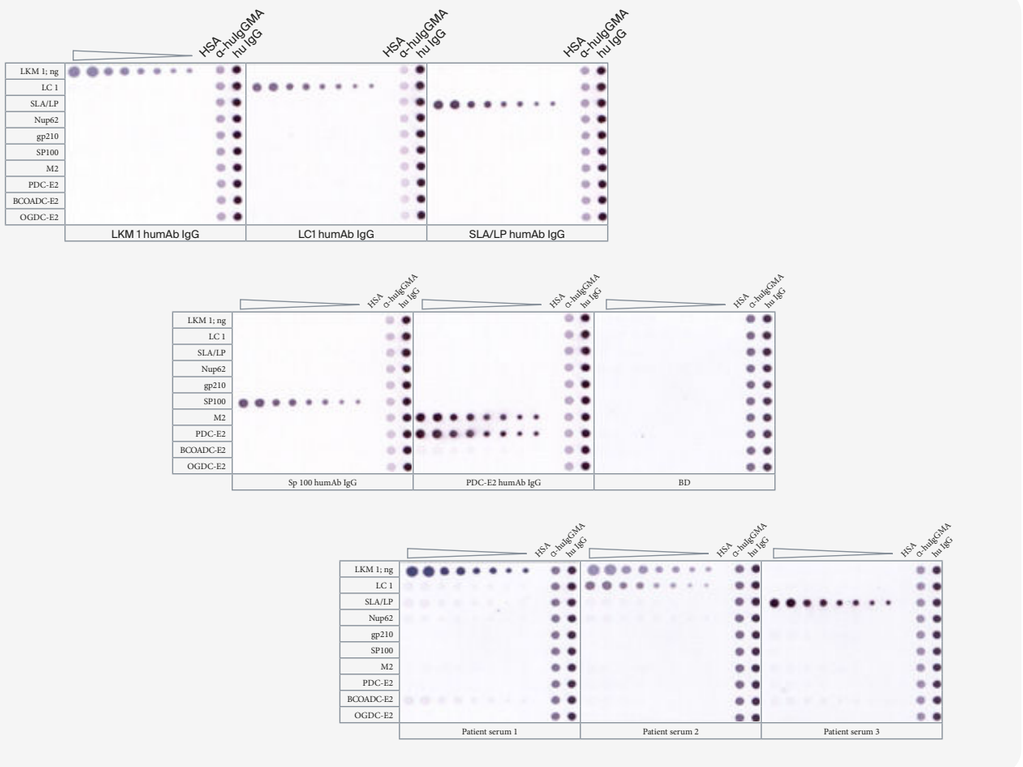
Anti-liver/kidney microsomal type 1 antibodies (anti-LKM 1) have been found to target Cytochrome P450 2D6 (CYP2D6) which is a member of a complex family of monooxygenases.

(Abuaf et al. 1992; Bourdi et al. 1990; Gonzales and Gelboin 1992; Gueguen et al. 1988)

CYP2D6 localizes to the Endoplasmic Reticulum (ER) where it is involved in hydroxilizing steroids, fatty acids and xenobiotic compounds (Gonzalez et al. 1992; Rizetto et al. 1974).

The International Autoimmune Hepatitis Group (IAIHG) has reported the presence of these autoantibodies to be a characteristic of autoimmune hepatitis (AIH) type 2 (Homberg et al. 1987; Liberal et al. 2014). Recombinant LKM 1 has enabled the establishment of immunoassays for a better analysis of the autoantibodies, which are reported to be potentially mixed up with anti-mitochondrial autoantibodies (AMA) in Indirect Immunofluorescence (IIF) (Bogdanos et al. 2003; Czaja et al. 1992). In addition, this recombinant antigen allows the differentiation of cytochrome P450 2D6 / LKM 1 autoantibodies from auto-antibodies against other monooxygenases of the P450 family, which is not possible in IIF.

Figure: Immunodot analysis of serum from a blood donor (BD; negative control) and patient sera (1-3). Besides new generation LKM 1; ng, LC 1 and SLA/LP the following antigens of anti-mitochondrial autoantibodies (AMA) were included: Nup62, gp210, Sp100, M2, BCOADC-E2, OGDC-E2, PDC-E2. Human serum albumin (HSA) served as a negative control, anti-human IgGMA (α-hu IgGMA) and human IgG (hu IgG) were used as positive controls. Samples were also probed with BBI's human chimeric monoclonal antibodies against LKM 1, LC 1, SLA/LP, PDC-E2.



Formiminotransferase cyclodeaminase is a bifunctional enzyme, which is involved in the metabolism of both histidine and the vitamine folate. Folate and its derivates are required for the synthesis of DNA, RNA and amino acids. (Mao et al. 2004).

Ordering information

Product description	Codes	Size
Cytochrome P450 2D6 (LKM 1; ng)	31800 31801	0.1 mg 1.0 mg
Formiminotransferase Cyclodeaminase (LC 1)	13700 13701	0.1 mg 1.0 mg
SLA/LP	30800 30801	0.1 mg 1.0 mg

Formiminotransferase cyclodeaminase is the antigen of liver cytosol antigen type 1 (LC 1) autoantibodies which are reported to be present in approximately 30% of AIH type 2 patients and to occur together with LKM 1 autoantibodies. (Lapierre et al. 1990; Muratori et al. 2001).

Although LC 1 autoantibodies give rise to a characteristic pattern in IIF, this pattern may be masked by concurrent LKM 1 autoantibodies. (Sebode et al. 2018). Therefore, using recombinant LC 1 in immunological assays may help to solve this limitation. Intriguingly, in approximately 10% of the patients, autoantibodies against LC 1 are reported to represent the only serological marker for AIH type 2 (Abuaf et al. 1992).

Cytosolic Soluble Liver Antigen / Liver Pancreas antigen (SLA/LP) is specifically detected in about 20% of the AIH patients. Target of anti-SLA/LP is a 50 kDa UGA serine tRNA-associated protein complex (tRNA(Ser)Sec). A high specificity and frequency (47.5%) of the anti-tRNP(Ser)Sec autoantibodies for severe forms of type 1 AIH has been shown (Costa et al. 2000; Wies et al. 2000).

BBI's AIH specific antigens are produced in either E. coli or the baculovirus/insect cell expression system.

References:
Abuaf et al. (1992) Hepatology. 16 (4): 892-898
Bogdanos et al. (2003) Clin Liver Dis. 7 (4): 759-777
Bourdi et al. (1990) J Clin Invest. 85 (6):1967-1973
Costa et al. (2000) Clin Exp Immunol. 121 (2): 364-374
Czaja et al. (1992) Gastroenterology. 103 (4): 1290-1295
Gonzales and Gelboin (1992) Environ Health Perspect. 98: 81-85
Gonzales et al. (1992) Tohoku J Exp Med. 168 (2): 67-72
Gueguen et al. (1988) J Exp Med. 168 (2): 801-806
Homberg et al. (1987) Hepatology. 7 (6): 1333-1339
Lapierre et al. (1999) Gastroenterology. 116 (3): 643-649
Liberal et al. (2014) Autoimmun Rev. 13 (4-5): 435-440
Mao et al. (2004) EMBO J. 23 (15): 2963-2971
Muratori et al. (2001) Hepatology. 34 (3): 494-501
Rizzetto et al. (1973) Clin Exp Immunol. 15 (3): 331-344
Rizzetto et al. (1974) Immunology. 26 (3): 589-601
Sebode et al. (2018) Front Immunol. 9: 609
Wies et al. (2000) Lancet. 355: 1510-1515

Celiac disease

Tissue Transglutaminase and Gliadin

Celiac Disease (CD) is a chronic gastrointestinal disorder most likely caused by an abnormal immune reaction to wheat gliadin and related gluten components from barley, rye, and possibly oats.

(Arentz-Hansen et al. 2000)

Originally thought to be predominantly diagnosed in populations of European origin, newer studies indicate that other regions of the world have similar diagnostic rates. Worldwide, CD exerts a prevalence of approximately 1%, although the numbers can vary between different countries (Lionetti and Catassi 2011).

The disease is characterized by flattening of the jejunal mucosa and intestinal lesions of variable severity in genetically predisposed individuals. CD does not fit the typical characteristics of an autoimmune disease; yet it is associated with the occurrence of autoantibodies. Dietary gluten induces the production of antibodies against gliadin and the human endogenous tissue transglutaminase (tTG). The diagnosis of CD involves the serological analysis for the presence of anti-tTG and anti-gliadin antibodies with the analysis of the former showing both a higher sensitivity and specificity. Furthermore, the levels of these autoantibodies appear to highly correlate with the activity and severity of the disease, and are therefore especially useful for the patient's dietary and therapeutic monitoring (Schuppan et al. 2013).

Traditionally, tTG isolated from guinea pig tissue has been used as the antigen in the development of diagnostic tests for CD. Since guinea pig and human tTG are only 80% identical, efforts were increased to produce recombinant human tTG (Gentile et al. 1991; Wong et al. 2002).

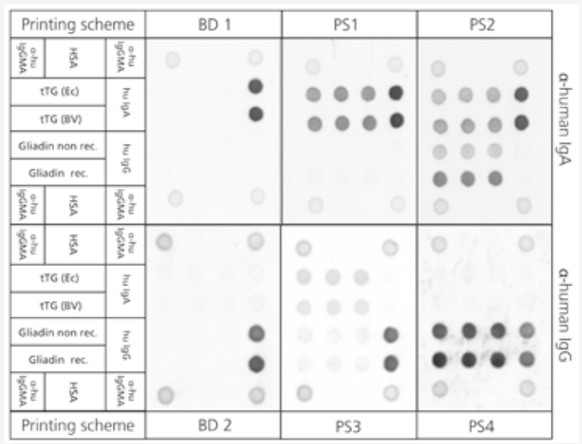


Figure: Immunodot analyses of sera from blood donors (BD1-2) and patients with celiac disease (PS1-4) for the presence of IgA (upper panel) and IgG autoantibodies (lower panel) directed against tissue transglutaminase (tTG) and/or gliadin. Antigens were spotted in triplicates on a nitrocellulose membrane as indicated. Human serum albumin (HSA) served as a negative control, anti-human IgGMA (α-hu IgGMA) and human IgG (hu IgG) were used as positive controls.

BBI's recombinant human tTG antigens have been specifically modified for improved handling: substitution of an amino acid within the enzymatic active site eliminates tTG's intrinsic protein cross-linking activity while maintaining its native three-dimensional structure and secondary GTPase activity.

Ordering information

Product description	Codes	Size
Tissue Transglutaminase (tTG; expressed in Baculovirus/Sf9)	15200 15201	50 µg 1.0 mg
Tissue Transglutaminase (tTG; expressed in E. coli)	14400 14401	50 µg 1.0 mg
Gliadin (recombinant; deamidated)	19500 19501	50 µg 1.0 mg
Gliadin (non recombinant)	31500 31501	50 µg 1.0 mg

This engineering assures reproducible properties of the recombinant antigen preparations by eliminating variable and ill-defined covalent aggregates between human tTG and host-cell proteins (Nurminskaya et al. 2012).

Two recombinant human tissue transglutaminase forms expressed in baculovirus/insect cells and E. coli, respectively, are available from BBI.

Monitoring of gliadin antibodies is recommended in pa-tients, who may test negative for anti-tTG autoantibodies, in the screening of populations at risk for CD, and other gluten-sensitive enteropathies (Schuppan et al. 2013).

BBI has successfully completed the recombinant protein approach by designing and producing a deamidated γ-gliadin isoform. Based on the sequence design, the epitopes present in this recombinant gliadin correspond to the deamidated neo-epitopes, which are formed in the natural gliadin antigen by transglutaminase-mediated glutamine side chain deamidation (Schwertz et al. 2004).

BBI's recombinant gliadin is produced in E. coli and non recombinant gliadin is isolated from wheat (Triticum aestivum) grain.

References:
Arentz-Hansen et al. (2000) Gut. 46 (1): 46-51
Gentile et al. (1991) J Biol Chem. 266 (1): 478-483
Leonard et al. (2014) Clin Exp Gastroenterol. 24: 25-37
Lionetti and Catassi (2011) Int Rev Immunol. 30 (4): 219-231
Nurminskaya et al. (2012) Int Rev Cell Mol Biol. 294: 1-97
Schuppan et al. (2013) Dtsch Arztebl Int. 110 (49): 835-846
Schwertz et al. (2004) Clin Chem. 50 (12): 2370-2375
Wong et al. (2002) J Clin Pathol. 55 (7): 488-494

Primary Biliary Cirrhosis (PBC)

Antigens associated with Primary Biliary Cirrhosis

Primary Biliary Cirrhosis (PBC) is a chronic and progressive autoimmune liver disease, which is characterized by the destruction of the bile ducts and portal inflammation leading to liver cirrhosis and consequently to hepatic failure.

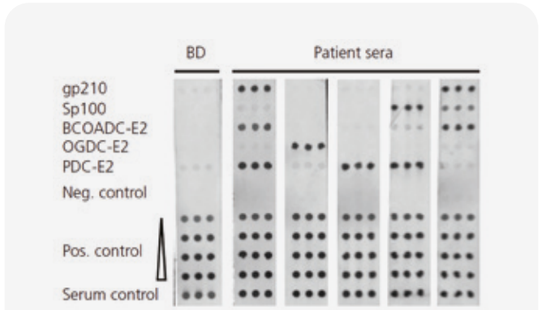


Figure: Immunodot analyses of the AMA antigens PDC-E2, OGDCE2 and BCOADC-E2, and ANA antigens Sp100 and gp210 using sera from a blood donor (BD) and primary biliary cirrhosis patients.

Serological PBC diagnosis is based on the detection of anti-mitochondrial autoantibodies (AMA) against the so-called M2 antigen, which can be found in over 90% of the patients. This antigen comprises the E2-subunits/ dihydrolipoamide transferases of three mitochondrial 2-oxo acid dehydrogenase complexes: pyruvate dehydrogenase complex (PDC-E2), 2-oxoglutarate dehydrogenase complex (OGDC-E2), and branched chain 2-oxo acid dehydrogenase complex (BCOADC-E2). All three E2-subunits are produced in the baculovirus/insect cell expression system and are available as separate parameters or as a mixture (M2), which contains equal masses of each protein.

Although AMA are an invaluable tool in the serological diagnosis of PBC, additional PBC-specific anti-nuclear autoantibodies (ANA) against nuclear autoantigens have been identified and can be detected in approximately 30-50% of the patients. Especially gp210 and Sp100 are of interest with autoantibodies against the former being found in approximately 25% of patients with M2/ AMA-positive PBC and up to 50% of those with M2/AMA-negative PBC. Autoantibodies against Sp100 are also found in approximately 25% of PBC patients and are considered a highly specific PBC marker. In contrast to M2 autoantibodies, these autoantibodies appear to be associated with disease progression and severity.

The evolutionary conserved nucleoporin gp210 is a transmembrane glycoprotein and part of the Nuclear Pore Complex (NPC), which regulates the transport between the nucleus and the cytoplasm.

Ordering information

Product description	Codes	Size
M2	18000 18001	50 µg 1.0 mg
BCOADC-E2	17700 17701	50 µg 1.0 mg
OGDC-E2	17800 17801	50 µg 1.0 mg
PDC-E2	17900 17901	50 µg 1.0 mg
Sp100	18900 18901	50 µg 1.0 mg
gp210	19000 19001	50 µg 1.0 mg
Nup62	19400 19401	50 µg 1.0 mg

Autoantibodies against gp210 create a rim like or membrane-like pattern (M-ANA) around the nucleus in indirect immunofluorescence (IIF). Nickowitz and Worman identified the epitopes to be localized within the cytoplasmic 58 residue C-terminal tail of gp210.

BBI has applied its recombinant protein technology to offer a variant of human gp210 comprising several repeats of the autoreactive cytoplasmic C-terminal tail. Sp100 is a nuclear protein with a deduced molecular weight of 55 kDa that is named after its speckled/multinuclear dots pattern (MND-ANA) observed in IIF assays and aberrant mobility at 100 kDa in protein gels. The cellular function of Sp100 is not well understood, but it appears to be involved in the regulation of gene transcription and the cellular response to viral infections.

As an auxiliary product, BBI offers a second recombinant human M-ANA antigen, the nucleoporin Nup62 that is directly involved in the molecular trafficking between the cytoplasm and the nucleus, and suggested to be involved in the centrosome homeostasis.

Nup62, Sp100, and gp210 are produced in the baculovirus/ insect cell expression system.

References:
Bogdanos et al. (2008) World J Gastroenterol. 14 (21): 3374-3387
Fusconi et al. (1991) Clin Exp Immunol. 83 (2): 291-297
Hohenester et al. (2009) Semin Immunopathol. 31 (3): 283-307
Nguyen et al. (2010) Best Pract Res Clin Gastro. 24 (5): 647-654
Nickowitz and Worman (1993) J Exp Med. 178 (6): 2237-2242

Crohn's disease

Glycoprotein 2 (GP2)

Crohn's disease (CD) and Ulcerative Colitis (UC) are the two most frequently occurring Inflammatory Bowel Diseases (IBD) in caucasians.

Mucosal inflammation in CD appears to occur when dysregulation of the immune system leads to an imbalance between tolerance to commensal microbiota or food-derived antigens and immunity to pathogens.

(Conrad et al. 2014)

The development of CD involves autoimmune mechanisms, and the occurrance of exocrine pancreas autoantibodies (PAB) in approximately 40% of CD patients is considered to be disease-specific. Interestingly, PAB have been detected in 68% of CD patients with extraintestinal complications such as idiopathic chronic pancreatitis.
(Somma et al. 2013; Stöcker et al. 1984).

PAB were first detected by Stöcker et al. in 1984 during a screen of 59 sera obtained from endoscopically and histologically confirmed CD patients. Using indirect immunofluorescence (IIF), 39% of all sera were found to contain PAB. In 2009, Roggenbuck et al. demonstrated for the first time that glycoprotein 2 (GP2) is the major autoantigen of CD-specific PAB.

GP2 is a highly glycosylated 78 kDa protein with N-linked carbohydrates. It accounts for up to 40% of all zymogen granule (ZG) membrane proteins in pancreatic acinar cells and is linked to the ZG membrane via a C-terminal glycosyl phosphoinositol (GPI) anchor (Hoops et al. 1993; Somma et al. 2013).

Upon hormonal or neuronal stimulation of the pancreas, GP2 is transported to the apical compartment of acinar cells. Following cleavage of its GPI anchor, GP2 is released into the pancreatic duct and subsequently into the intestinal lumen. As a self-binding glycoprotein, GP2 forms soluble aggregates in the pancreatic juice (Rindler et al. 1990).

The physiological function of GP2 in the pancreas is still elusive, but it has been suggested to be involved in the formation of pancreatic granules

(Colomer et al. 1994; Somma et al. 2013).

Ordering information

Product description	Codes	Size
GP2	19600 19601	50 µg 1.0 mg

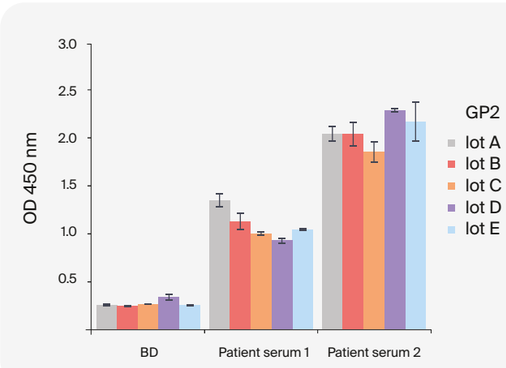


Figure: Analyses of sera from a healthy blood donor (BD) and patients with Crohn's Disease (Patient serum 1-2) for the presence of anti-GP2 autoantibodies by ELISA. Five different lots of recombinant human GP2 were used as coating antigen.

A major step forward in the understanding of CD has been the finding that GP2 is also expressed on the apical membrane of microfold/membranous cells (M cells). M cells are phagocytotic cells found in the Peyer's patches of the intestinal follicle-associated epithelium, which take up macromolecules and microbes from the intestinal lumen to induce the mucosal immune response. In addition, some results appear to indicate an immunosuppressive and anti-inflammatory role of GP2, which would further highlight its pivotal role in the regulation of the mucosal immune response (Hase et al. 2009).

Anti-GP2 autoantibodies constitute novel disease-specific markers, the quantification of which could improve the serological diagnosis of IBD. Detection of anti-GP2 autoantibodies by ELISA is a readily available and robust method for the assessment of CD-specific autoantibodies. In two studies carried out by (Roggenbuck et al. 2009, 2011), the level of GP2 specific autoantibodies in PAB positive and negative sera were analyzed by ELISA. In these studies, the levels of GP2 autoantibodies were reported to be significantly higher in PAB positive sera than in the controls and sera of UC patients. This seems to make the use of GP2 autoantibodies a useful serological tool to diagnose CD.

Recombinant human GP2 from BBI is produced in the baculovirus/insect cell expression system.

References:
Colomer et al. (1994) EMBO J. 13 (16): 3711-3719
Conrad et al. (2014) Autoimmun Rev. 13 (4-5): 463-466
Hase et al. (2009) Nature. 462 (7270): 226-230
Hoops et al. (1993) J Biol Chem. 268 (34): 25694-25705
Rindler et al. (1990) Eur J Cell Biol. 53 (1): 154-163
Roggenbuck et al. (2009) Gut. 58 (12): 1620-1628
Roggenbuck et al. (2011) Clin Chem Acta. 412 (9-10): 718-724
Roggenbuck et al. (2014) Clin Chem Lab Med. 52 (4): 483-494
Somma et al. (2013) Gastroenterol Res Pract. 2013: 683824
Stöcker et al. (1984) Dtsch Med Wochenschr. 109 (51-52): 1963-1969

BBI

Get in touch to arrange a sample or find out more about any of our products and services

info@bbisolutions.com
Int: +44 (0)1495 363 000
USA: +1 888 222 4149
China: +86 (0)21 6104 2216

Advancing health, together.

BBISOLUTIONS.COM