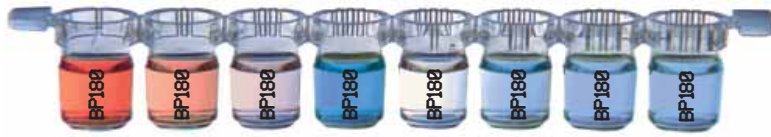




## Anti-BP180-4X ELISA (IgG)



**Indications:** Test system for the in vitro determination of antibodies against BP180 in human serum or plasma for the diagnosis of the following diseases: bullous pemphigoid, pemphigoid gestationis, mucous membrane pemphigoid and lichen ruber pemphigoides.

**Clinical significance:** Bullous autoimmune dermatoses belong to organ-specific autoimmune diseases. They are characterised by the formation of autoantibodies against structure proteins of the skin. These structural proteins establish the cell-to-cell contact in keratinocytes within the epidermis and the adhesion of the epidermis to the dermis. Bullous autoimmune dermatoses are divided in 4 main groups by means of their target antigens and the localisation of the blisters: pemphigoid and pemphigus diseases, epidermolysis bullosa acquisita and Dühring's dermatitis herpetiformis. In pemphigus diseases the blisters are formed intraepidermally, whereas they occur in all other bullous autoimmune dermatoses subepidermally.

With 0.7 to 1.8 new cases per year per 100,000 inhabitants, the bullous pemphigoid (BP) is the most frequent subepidermal blister-forming autoimmune dermatosis. The disease mainly affects elderly people. The manifestation of the BP are bulging blisters at the integument. However, the BP may proceed without blisters for weeks or months. Therefore, elderly patients with irritating skin disorders persisting for long periods should be tested for BP in differential diagnosis. Various immunological methods are used for differential diagnosis of the disease. The presence of circulating autoantibodies is significant in 90% of BP patients. These autoantibodies are mainly directed against 2 hemidesmosomal proteins. The BP antigens have molar masses of 180 kDa (BP180) and 230 kDa (BP230), respectively. BP230 is localised intracellularly in the hemidesmosomal plaque. BP180 is a transmembrane glycoprotein with an intracellularly localised C-terminus and an extracellular N-terminus. The ectodomain consists of 15 collagenous and 16 non-collagenous domains. The 16<sup>th</sup> non-collagenous domain (NC16A) directly flanking the keratinocyte membrane presents the immunogenic epitope. The majority of BP patients have autoantibodies against BP180. Direct and indirect immunofluorescence is used for the determination of autoantibodies. Tissue-bound autoantibodies and/or complement deposits in biopsies of perilesional skin can be determined using direct immunofluorescence. Circulating autoantibodies in the patient serum can be found by means of indirect immunofluorescence (substrate: oesophagus, primate and human skin). Autoantibodies against basement membrane show a fine linear staining between the stratum basale and the connective tissue. Autoantibody specificity can be characterised using monospecific ELISA or immunoblots.

Panel	n	Anti-BP180 positive
Bullous pemphigoid (BP)	118	106 (89.8%)
Pemphigoid gestationis (PG)	20	20 (100.0%)
Asymptomatic blood donors	494	10 (2.0%)
Rheumatoid arthritis (RA)	107	2 (1.9%)
Progressive systemic sclerosis (PSS)	50	2 (4.0%)
Systemic Lupus erythematosus (SLE)	72	1 (1.4%)
Sensitivity	118	89.8%
Specificity	723	97.9%

**Application of the Anti-BP180-4X ELISA:** The detection of autoantibodies in the skin and/or in the serum of patients is decisive in BP diagnosis. BP patients mainly have autoantibodies against BP180. These autoantibodies can be found in the skin using direct immunofluorescence. Autoantibodies circulating in the serum can be detected by means of indirect immunofluorescence using organ tissues. The Anti-BP180-4X ELISA<sup>1</sup> uses a tetramer of the immunogenic NC16A domain and is a reliable alternative for the indirect immunofluorescence test. The advantage of the ELISA is the clear characterisation of the autoantibody specificity when using the recombinant BP180 and the resulting differentiation of other bullous autoimmune dermatoses such as pemphigus diseases, epidermolysis bullosa acquisita and Dühring's dermatitis herpetiformis. The multimer form of the autoantigen increases the immunoreactivity, thus improving the efficiency of the autoantibody test. Unlike the titer in indirect immunofluorescence, the serum level of autoantibodies against BP180 correlates with the BP activity. The detection is therefore helpful in the evaluation of the disease activity before and after therapy.

<sup>1</sup> German Patent Application No. 10 2006 059 574.2

### EUROIMMUN Microplate ELISA

**Autoantibody determination:**  
AMA M2-3E (IgG)  
ANCA Profile (IgG)  
ANA Screen (IgG)  
ANA Screen 9\* or 11\* (IgG)  
ANA VarioProfile (IgG)  
BP180-4X (IgG)  
C1q (IgG)  
cardiolipin (IgA, IgG, IgM, IgAGM)  
circulating immune complexes (CIC)  
cyclic citrullinated peptide (CCP; IgG)  
centromere protein B (IgG)  
double-stranded DNA (dsDNA, nDNA; IgG)  
dsDNA-NcX (IgG)  
ENA Pool\* (IgG)  
ENA PoolPlus (IgG)  
ENA ProfilePlus 1 or 2 (IgG)  
ENA SLE Profile 1 or 2 (IgG)  
GAD  
GAD/IA-2 Pool  
glomerular basement membrane (GBM; IgG)  
β2-glycoprotein 1 (IgA, IgG, IgM, IgAGM)  
histones (IgG)  
IA-2  
intrinsic factor (IgG)  
Jo-1 (IgG)  
liver cytosolic antigen type 1 (LC-1; IgG)  
liver-kidney microsomes (LKM-1; IgG)  
myeloperoxidase (MPO; IgG)  
nRNP/Sm (IgG)  
nucleosomes (IgG)  
p53 (IgG)  
parietal cells (PCA; IgG)  
PM-Scl (PM-1; IgG)  
phosphatidylserine (IgA, IgG, IgM, IgAGM)  
proteinase 3 (IgG)  
PR3 hn-hr (IgG)  
PR3 capture (IgG)  
rheumatoid factor (IgA, IgG, IgM)  
ribosomal P-proteins (IgG)  
Scl-70 (IgG)  
single-stranded DNA (ssDNA; IgG)  
SLA/LP (IgG)  
Sm (IgG)  
SS-A (Ro; IgG)  
SS-B (La; IgG)  
thyroglobulin (TG; IgG)  
thyroid peroxidase (TPO; IgG)  
tissue transglutaminase (endomy; IgA, IgG)  
TSH receptor (TBI; IgG)  
TRAK Fast (IgG)

**Further autoimmune diagnostics:**  
GAF-3X (IgA, IgG)  
gliadin (IgA, IgG)  
Saccharomyces cerevisiae (IgA, IgG)

**Infectious serology:**  
Adenovirus (IgA, IgG, IgM)  
Borrelia (IgG, IgM)  
Borrelia ViSE (IgG)  
Chlamydia pneumoniae (IgA, IgG, IgM)  
Chlamydia trachomatis (IgA, IgG, IgM)  
Cytomegalovirus (IgG, IgM)  
Diphtheria toxoid (IgG)  
Epstein-Barr virus capsid ag (IgA, IgG, IgM)  
Epstein-Barr virus early ag (IgA, IgG, IgM)  
Epstein-Barr virus nuclear ag, EBNA-1 (IgG)  
Helicobacter pylori (IgA, IgG)  
Helicobacter pylori CagA (IgA, IgG)  
HSV-1 (glycoprotein C1; IgA, IgG, IgM)  
HSV-2 (glycoprotein G2; IgA, IgG, IgM)  
HSV-1/2 Pool (IgA, IgG, IgM)  
Influenza virus type A (IgA, IgG, IgM)  
Influenza virus type B (IgA, IgG, IgM)  
Legionella pneumophila (IgA, IgG, IgM)  
Measles virus (IgG, IgM)  
Mumps virus (IgG, IgM)  
Mycoplasma pneumoniae (IgA, IgG, IgM)  
Parainfluenza virus Pool (IgA, IgG, IgM)  
RSV (IgA, IgG, IgM)  
Rubella virus (IgG, IgM)  
SARS-CoV (IgG)  
TBE virus (IgG, IgM)  
Tetanus toxoid (IgG)  
Toxoplasma gondii (IgG, IgM)  
Treponema pallidum (IgG, IgM)  
Varicella zoster virus (IgG, IgM)  
Yersinia enterocol. virulence fact. (IgA, IgG)

**Allergology:**  
total IgE  
Allercoat™ 6-ELISA (600 different allergens and allergen mixtures)

**Serum proteins and tumour markers:**  
anti-p53

\* Currently not available as IVD in the EU.

Made in Germany



**Autoantibody determination:**

**EUROASSAY:**

flexible profiles of up to 7 antigens from:

ENA and related antigens: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, dsDNA, histones, nucleosomes, CENP B, PM-Scl, ribosomal P-proteins, AMA M2

liver antigens: LKM-1, LC-1, SLA/LP, AMA M2, M4, M9

ANCA antigens: MPO, PR3

thyroid antigens: TG, TPO

**EUROLINE:**

ANA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, CENP B, dsDNA, nucleosomes, histones, ribosomal P-proteins

ANA Profile 3: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA M2

Anti-ENA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1

Myositis Profile: Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12, Ro-52

Liver Profiles: AMA M2, 3E (BPO), Sp100, PML, gp210, LKM-1, LC-1, SLA/LP, Ro-52

Neuronal Antigens Profile: amphiphysin, CV2/CRMP5, PNMA2 (Ma-2), Ri, Yo, Hu

Anti-Ganglioside Profile 1: GM1, GD1b, GQ1b

Anti-Ganglioside Profile 2: GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b

ANCA Profiles: MPO, PR3, GBM

**EUROLINE-WB:**

liver-specific antigens (+ recomb. SLA/LP)  
neuronal antigens (+ recomb. Hu, Yo, Ri)  
HEP-2 cell antigens (+ SS-A and Ro-52, CENP B)  
Myositis ag (Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12)

**Infectious serology:**

**EUROLINE:**

EBV Profile (IgG, IgM, VCA gp125, VCA p19 and EBNA-1, p22, EA-D)  
TORCH Profile\* (T. gond., rubella, CMV, HSV-1, -2)  
Malaria Profile 1: Plasmodium falciparum HRP-2 and MSP-2, Plasmodium vivax MSP and CSP

**Westernblot:**

Borrelia burgdorferi (IgG, IgM)  
Borrelia afzelii (IgG, IgM)  
Borrelia garinii (IgG, IgM)  
Epstein-Barr virus (IgG, IgM)  
Helicobacter pylori (IgA, IgG)  
Treponema pallidum (IgG, IgM)  
Yersinia enterocol. virulence fact. (IgA, IgG)

**EUROLINE-WB:**

Anti-Borrelia (B. afzelii + rec. VlsE)  
Anti-HSV (HSV-1 + HSV-2 gG2)  
Treponema pallidum + cardiolipin

**Allergy:**

**EUROASSAY:**

Domestic Animal Profile (IgE)  
Food Profile (IgE)  
Inhalation Profile (IgE)  
Insect/Venom Profile (IgE)  
Latex Profile (IgE)  
Latex plus Profile (with ficus and fruit; IgE)

**EUROLINE:**

Atopy Profile (IgE)  
Food Profile (IgE)  
Inhalation Profile (IgE)  
Paediatric Inhalation Profile  
Pollen-Food Cross Reaction Profile (IgE)

**Software/Automation:**

EUROLineScan  
camera system EUROBlotCamera  
scanner system EUROBlotScanner  
incubation processor EUROBlotMaster

**EUROIMMUN**

**Radioimmunoassays**

**Autoantibody determination:**

thyroid peroxidase (TPO; IgG)  
thyroglobulin (TG; IgG)  
TSH receptor (IgG)  
acetylcholine receptor (AChR; IgG)  
glutamic acid decarboxylase (GAD; IgG)  
insulin (IAA; IgG)  
P/Q calcium channel\* (VGCC; IgG)  
tyrosine phosphatase (IA2; IgG)  
dsDNA (IgA/IgG/IgM)

**Antigen determination:**

thyroglobulin (TG)

**Hormone determination:**

free triiodothyronin (FT3)  
free thyroxin (FT4)  
thyrotropin (TSH)  
calcitonin

\* Currently not available as IVD in the EU.

**Made in Germany**

Version: 02/07  
EA\_1502\_D\_UK\_A01

## Test Characteristics Anti-BP180-4X ELISA (IgG)

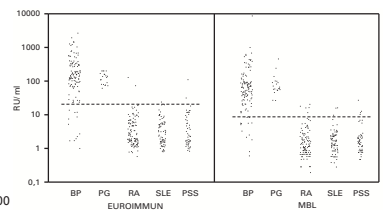
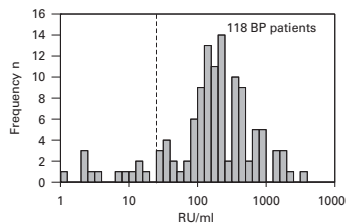
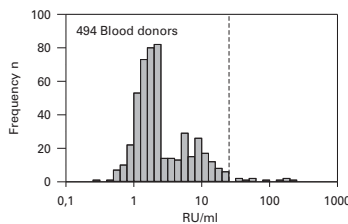
**Linearity:** The linearity of the ELISA was determined by assaying serial dilutions of 6 serum samples. The linear regression was calculated, R<sup>2</sup> amounting to > 0.95 in all samples. The Anti-BP180-4X ELISA (IgG) is linear at least in the range of 10 to 199 RU/ml.

**Reproducibility:** The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 4 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Serum	Intra-assay variation, n = 20		Inter-assay variation, n = 4 x 6	
	Mean value (RU/ml)	CV (%)	Mean value (RU/ml)	CV (%)
1	27	3.0	26	4.8
2	61	1.2	61	3.1
3	119	1.6	119	4.2
4	177	2.1	174	2.6

**Clinical sensitivity and specificity:** Sera from 118 BP patients, a control panel of 229 patients with other autoimmune diseases and 494 healthy blood donors were investigated using the EUROIMMUN Anti-BP180-4X ELISA. The sensitivity of the ELISA for BP was 89.8%, with a specificity of 97.9%.

**Reference range:** Levels of anti-BP180 antibodies were determined in 494 sera from healthy blood donors of between 18 and 68 years of age (185 women, 309 men) using the EUROIMMUN ELISA. The mean concentration of antibodies against BP180 was 4.5 RU/ml and the results ranged from 0.01 to 168.0 RU/ml. With a cut-off of 20 RU/ml, 2.0% of blood donors were anti-BP180 positive.



**ROC analysis:** In an analysis of 118 BP samples and 723 control sera the following results were achieved:

**Correlation of the EUROIMMUN and MBL Anti-BP180 ELISA:** The antibody concentration was determined in 118 sera of BP patients using the EUROIMMUN and MBL Anti-BP180-ELISA. The qualitative results of the ELISA correlated in 99%.

EUROIMMUN	MBL	
	positive	negative
positive	105	1
negative	0	12

Blood donors, n = 494		
Percentile	95 <sup>th</sup>	98 <sup>th</sup>
Cut-off	12.5 RU/ml	27.4 RU/ml

Cut-off	Specificity	Sensitivity
13.4 RU/ml	95%	91%
19.8 RU/ml	98%	90%
MBL: 9.0 RU/ml	95%	89%

<sup>1</sup> according to the guidelines of the manufacturer  
<sup>2</sup> determined in 118 BP sera and 229 control sera

**Technical data:**

Antigen

Tetramer of the immunogenic NC16A domain (BP180), based on human cDNA, expressed in *E.coli*

Calibration

Quantitative, in relative units per milliliter (RU/ml).  
Calibration serum 1: 200 RU/ml  
Calibration serum 2: 20 RU/ml ; cut-off  
Calibration serum 3: 2 RU/ml

Sample dilution

Serum or plasma; 1 : 101 in sample buffer.

Reagents

Ready for use. Exception: wash buffer (10x). Colour-coded solutions, largely exchangeable with those of other EUROIMMUN-ELISA.

Test procedure

30 min / 30 min / 15 min. Room temperature. Fully automatable.

Measurement

450 nm. Reference wavelength between 620 nm and 650 nm.

Kit format

48 single break-off wells, incl. all necessary reagents.

Order no.

EA 1502-4801-2 G