



Anti-BP230-CF ELISA (IgG)



Indications: Test system for the in vitro determination of antibodies against BP230 in human serum or plasma for the diagnosis of the following diseases: bullous pemphigoid (BP), pemphigoid gestationis (PG), linear IgA dermatosis (LAD), mucous membrane pemphigoid and lichen ruber pemphigoides.

Clinical significance: Bullous autoimmune dermatoses belong to the organ-specific autoimmune diseases. They are characterised by the formation of autoantibodies against structural 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 into 4 main groups based on 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 in all other bullous autoimmune dermatoses they occur subepidermally.

With an incidence of 1.3 to 4.2 new cases per year per 100,000 inhabitants, bullous pemphigoid is the most frequent subepidermal blister-forming autoimmune dermatosis in central Europe and north America. The disease mainly affects elderly people. The manifestation of BP is bulging blisters at the integument. However, 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. Autoantibodies in BP are mainly directed against 2 hemidesmosomal proteins: BP180 (type XVII collagen) and BP230. **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 16th non-collagenous domain (NC16A) flanking the keratinocyte membrane presents the immunogenetic epitope. The majority of BP patients have autoantibodies against BP180-NC16A. BP180-NC16A is, moreover, the immunodominant region in patients with PG and Lichen planus pemphigoides and is recognised in around half of patients with mucosal pemphigoid and antibodies against BP180. **BP230** is a glycoprotein of the hemidesmosomal inner cytoplasmic plaque and contributes to the anchoring of the keratin filament system via its C-terminal domain. The N-terminal end of BP230 is important for its integration into the hemidesmosomes and interacts with BP180 and the $\beta 4$ -subunit of $\alpha 6\beta 4$ -integrin. 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.

Application of the Anti-BP230-CF 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. The Anti-BP230-CF ELISA supplements the serological diagnosis of BP via the detection of a second, independent autoantigen in the genetic sense. Anti-BP230 develop with a time lag compared to Anti-BP180. The parallel determination of autoantibodies against BP180 and BP230 increases the serological hit rate in the diagnosis of bullous pemphigoid. The serum level of autoantibodies against BP180 correlates with the disease activity of BP, the serum level of autoantibodies against BP230 with the duration of the disease.

Panel	n	Anti-BP230-CF ELISA
Bullous pemphigoid (BP)	118	67 (56.8%)
Pemphigoid gestationis (PG)	20	1 (5.0%)
Asymptomatic blood donors	483	10 (2.1%)
Rheumatoid arthritis (RA) and other arthritides	170	3 (1.8%)
Systemic Lupus erythematosus (SLE)	56	5 (8.9%)
Linear IgA dermatosis (LAD)	20	1 (5.0%)
Sensitivity for bullous pemphigoid	118	56.8%
Specificity for bullous pemphigoid	729	97.4%

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-NC16A-4X (IgG)
 BP230-CF (IgG)
 C1q (IgG)
 cardiolipin (IgA, IgG, IgM, IgAGM)
 circulating immune complexes (CIC)
 cyclic citrullinated peptide (CCP; IgG)
 centromere protein B (IgG)
 desmoglein 1 (IgG)
 desmoglein 3 (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)
 $\beta 2$ -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)
 Sa (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:
 gliadin (GAF-3X; 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)
 Parvovirus B19 (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



EUROIMMUN Immunoblots

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, SLALP, 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

ANA Profile 5: nRNP/Sm, Sm, RNP70, RNPA, RNPc, 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

Systemic Sclerosis Profile: Scl-70, CENP A, CENP B, RP11, RP155, Fibrillarin, NOR90, Th/To, PM-Scl100, PM-Scl75, Ku, PDGFR, Ro-52

Myositis Profile 3: Mi-2, Ku, PM-Scl100, PM-Scl75, SRP, Jo-1, PL-7, PL-12, OJ, EJ, Ro-52

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

Neuronal Antigens Profile 2: amphiphysin, CV2.1** PNMA2 (Ma-2/Ta), 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:

neuronal antigens (+ recomb. Hu, Yo, Ri)
HEp-2 cell antigens (+ SS-A and Ro-52, CENP B)

Infectious serology:

EUROLINE:

Bordetella pertussis (IgA, IgG)
Borrelia-RN-AT (p18, p19, p20, p21, p58, OspC, p39, p83, LbB, LbA, VisE Bg, VisE Bb, VisE Ba)
EBV Profile (IgG, IgM, VCA gp125, VCA p19 and EBNA-1, p22, EA-D)
Hanta virus (IgG, IgM)
TORCH Profile* (T. gond., rubella, CMV, HSV-1, -2)

Westembloit:

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

EUROLINE-WB:

Anti-Borrelia (B. afzelii + rec. VisE)
Anti-HSV (HSV-1 + HSV-2 gG2)
Helicobacter pylori (IgA, IgG)
Treponema pallidum + cardiolipin

Allergology:

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 triiodothyronine (FT3)
free thyroxine (FT4)
thyrotropin (TSH)
calcitonin

* Currently not available as IVD in the EU.
** CV2 partial protein, which only contains the N-terminally localised epitopes of the antigen.

Made in Germany

Version: 04/10
EA_1502_D_UK_B02

Test Characteristics Anti-BP230-CF ELISA (IgG)

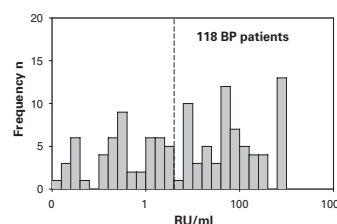
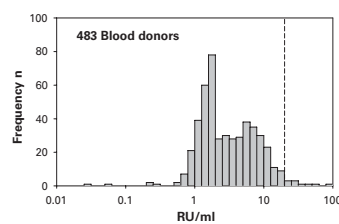
Linearity: The linearity of the ELISA was determined by assaying 4 serial dilutions of 6 serum samples. The linear regression R^2 was >0.95 for all samples. The Anti-BP230-CF ELISA (IgG) is linear at least in the range of 17 to 190 RU/ml

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	24	5.0	23	6.8
2	61	4.6	60	3.5
3	97	3.4	101	6.1
4	114	3.0	111	3.4

Clinical sensitivity and specificity: Sera from 118 BP patients, 20 PG patients, a control panel of 246 patients with other autoimmune diseases (RA, SLE, LAD) and 483 healthy blood donors were investigated using the EUROIMMUN Anti-BP230-CF ELISA. The sensitivity of the ELISA for BP was 57%, with a specificity of 97%. In the PG panel 5% of patients were positive.

Reference range: Levels of anti-BP230 antibodies were analysed in 483 sera from healthy blood donors of between 18 and 67 years of age (177 women, 306 men) using the EUROIMMUN Anti-BP230-CF ELISA. The mean concentration of antibodies against BP230 was 4.9RU/ml and the values ranged from 1.0 to 94.5RU/ml. With a cut-off of 20RU/ml, 2.1% of the blood donors were anti-BP230 positive.



Blood donors, n = 483		
Percentile	95 th	98 th
Cut-off	14.1 RU/ml	20.4 RU/ml

ROC analysis: In an analysis of 118 BP samples and 729 control sera the following results were achieved:

Cut-off	Specificity	Sensitivity
16.0RU/ml	95%	61%
23.6RU/ml	98%	56%

Correlation with the EUROIMMUN Anti-BP180-NC16A-4X ELISA: 118 sera from BP patients were tested with the EUROIMMUN Anti-BP230-CF ELISA and the Anti-BP180-NC16A-4X ELISA. By combining both ELISAs the sensitivity for BP amounted to 93%.

EUROIMMUN Anti-BP230-CF ELISA (IgG)		EUROIMMUN Anti-BP180-NC16A-4X ELISA (IgG)	
		positive	negative
pos.	62	5	
neg.	43	8	

Technical data:

Antigen

BP230 is a biochemically purified C-terminal piece of the human "230 kDa bullous pemphigoid antigen" 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, with the exception of the wash buffer (10x). Colour-coded solutions, in most cases exchangeable with those in other EUROIMMUN ELISA kits.

Test procedure

30 min / 30 min / 15 min. Room temperature.

Measurement

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

Test kit format

48 break-off wells. Kit includes all necessary reagents.

Order number

EA 1502-4801-1 G