

**BPI IgG ELISA Kit**

Quantitative/qualitative assay for bactericidal permeability  
increasing protein IgG antibodies

**BPI IgA ELISA Kit**

Semi-quantitative/qualitative assay for bactericidal permeability  
increasing protein IgA antibodies

Product code GD05 &amp; GD11 96 tests

For *in vitro* research use only

140108

**1. Intended use**

The BPI IgG and IgA kits are rapid ELISA methods for the detection of IgG and IgA antibodies to neutrophil cytoplasmic bactericidal permeability increasing protein (BPI). The components of the kit are for *in vitro* research use only.

**2. Explanation of the Test**

BPI is a constituent of the azurophilic granules of neutrophils. It is a highly cationic, 55 kD membrane-associated, cytotoxic protein found only in cells of the myeloid series. The potent toxicity of BPI is directed exclusively at gram-negative bacteria.

A substantial number of serum samples give positive c-ANCA or p-ANCA staining patterns by indirect immunofluorescence but are negative when tested for MPO and PR-3 antibody activity. Such samples can often be shown to have activity against BPI. BPI is an important antigen in vasculitis and may reflect the involvement of gram-negative bacteria in the aetiology of this disease. BPI IgG and BPI IgA levels are increased in bacterial infections involving the lungs such as in cystic fibrosis. BPI IgA levels closely correlate with lung function in such patients.

**3. Principle of the test**

Diluted serum samples are incubated with purified BPI immobilised on microtitre wells. After washing away unbound serum components, rabbit anti-human IgG or IgA conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the standards, positive control and samples are measured using a microplate reader at 450nm. Optical density is directly proportional to antibody activity in the sample.

**4. Materials included in the kit**

- **Microplate:** 96 wells in 12 X 8 break-apart strips, pre-coated with purified BPI, with holder in a foil bag with desiccant.
- **Reagent 1: Sample Diluent** 10 mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 100ml, (blue), ready to use
- **Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concentrate** (x10)
- **Reagent 3: Conjugate** rabbit anti-human IgG (red) or IgA (yellow) conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12 ml, ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standards: IgG** - 3.1, 6.2, 12.5, 25, 50 & 100U/ml; **IgA** - 0 & 10 U/ml, 1ml of 10mM Tris-buffered saline containing human serum IgG/IgA antibodies to BPI, ready to use
- **Positive control:** 1ml of 10mM Tris-buffered saline containing human serum antibodies to BPI, ready to use (IgG), lyophilised (IgA)
- **Instructions for use**

**5. Other equipment required**

1. Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable automated system may be used.
2. Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

**6. Precautions****6.1 Safety Precautions**

1. All reagents in this kit are for research use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. All human source material used in the preparation of standards and control for this product have been tested and found negative for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the TMB Substrate Solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local legislation.

**6.2 Technical Precautions**

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 – 8°C after use.
3. Include the Positive Control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
7. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and the control into the wells without delay. Therefore ensure that all samples are ready to dispense.

**7. Shelf life and storage conditions**

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Store the reconstituted Positive Control at -20°C (IgA only). Do not freeze any other kit component. The diluted Wash Buffer and Sample Diluent have a shelf life of 3 months if stored in a closed bottle at 2 – 8°C.

**8. Specimen collection and storage**

Serum samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

**9. Preparation of reagents**

1. Dilute the Wash Buffer (**Reagent 2**) 1: 9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.
2. Reconstitute the Positive Control in 1 ml of distilled water (IgA only)

#### 10. Assay Procedure

1. Dilute patient samples 1:50 in Sample Diluent (e.g. 20µl serum plus 1ml diluent).
2. Assemble the number of strips required for the assay.
3. For quantitative assays (IgG only), dispense 100 µl of Sample Diluent as the 0 U/ml Standard. Dispense each Standard, the Positive Control and the diluted patient samples into appropriate wells.

For semi-quantitative assays (IgA only), dispense the 0 and 10 U/ml Standards, together with the Positive Control and diluted patient samples.

For qualitative assays, dispense only the 3.1 U/ml Standard (IgG assays) or the 10 U/ml Standard (IgA assays), together with the Positive Control and diluted samples.

4. Incubate for **60** minutes at room temperature.
5. After 60 minutes, decant or aspirate the well contents and wash the wells 3 times automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

##### Manual Wash Procedure:

Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with Wash Buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.

6. Dispense 100µl of Conjugate (**Reagent 3**) into each well. Incubate the wells for **30** minutes at room temperature.
7. After 30 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.
8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate (**Reagent 4**) into each well. Incubate the plate for **10** minutes.
9. Add 100µl of Stop Solution (**Reagent 5**) to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.
10. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

#### 11. Quality control

The expected OD values and the acceptance ranges for the Standards and the Positive Control are given on the certificate included in the kit.

The control is intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

#### 12. Interpretation of Results

##### Quantitative results (IgG)

Plot the OD of each standard against its concentration and draw the best-fit curve through the points. Read the unknowns off this curve. Values above 4 U/ml are significantly elevated.

##### Semi-quantitative results (IgA)

Plot the OD of the 0 and 10 U/ml Standards against their respective concentrations and draw a straight line through the points. Read the unknowns off this line. Samples with optical densities greater than 10 U/ml are positive for anti-BPI IgA.

##### Qualitative results

IgG – Samples with ODs greater than the 3.1 U/ml Standard are positive for anti-BPI IgG.

IgA - Samples with ODs greater than the 10 U/ml Standard are positive for anti-BPI IgA.

#### 13. Limitations of the Procedure

BPI IgA and IgG ELISA results should be used in conjunction with other test results and overall clinical presentation.

#### 14. Reproducibility

Within assay imprecision: < 12%  
Between assay imprecision: <12%

##### **Method Summary**

- Dilute sera 1:50 with Sample Diluent (**Reagent 1**)
- Dispense Standards, the Positive Control and the diluted sample into the microplate wells
- Incubate for **60** minutes at room temperature.
- Wash the wells three times
- Dispense 100µl of Conjugate (**Reagent 3**) into each well
- Incubate at room temperature for **30** minutes
- Wash the wells four times
- Add 100µl of TMB Substrate (**Reagent 4**) to each well
- Incubate at room temperature for **10** minutes
- Add 100µl Stop Solution (**Reagent 5**) to each well
- Read the optical density at 450nm (single wavelength) or 450/620nm (dual wavelength).

##### **Further reading**

- Zhao, M H, et al Clin Exp Immunol 1995; 99, 49-56  
 Zhao, M H, et al Q J Med 1996; 89,259-265  
 Stoffel M P, et al Clin Exp Immunol 1996; 104, 54-59  
 Weiss, J et al Blood 1987; 69,652-659  
 Savage, C O S et al, Lancet 1987 1:1389-1393  
 Ooi, C I et al, J Exp Med 1991; 174, 649-655  
 Jeanette J C et al, Arthritis Rheum 1994, 37, 187-192  
 Guillevin F., et al J Rheumatol 1993, 20(8) 1345-1349  
 Ronda N, et al Clin Exp Immunol 1994, 95 49-55  
 Mulder A H, et al Arthritis Rheumatol 1993,36(8) 1054-1060  
 Wiik, A et al, Manual of Biological Markers of Disease 1993; A9: 1-14  
 Mulder A H, et al Clin Exp Immunol 1994, 95(3) 490-497  
 Broekroelofs J, et al. Dig Dis Sci 1994, 39(3) 545-549  
 Pokorny C S, et al J Gastroenterol Hepatol 1994, 9(1) 40-44  
 Robinson A J, *Nephro Dial Transplant* 1994 9, 119-126  
 Gross W L, et al *Clin Exp Immunol* 1993, 93 (Suppl 1) 7 - 11  
 Sediva A. et al (1996) Sarcoidosis Vasculitis and diffuse lung diseases. (7<sup>th</sup> International ANCA Workshop) 13,(3) 275