

immuno
Diagnostik

Apotech[®]

BAFF, Soluble (human) ELISA Kit

*For the in vitro determination of BAFF soluble (human) in
serum and cell culture supernatant*

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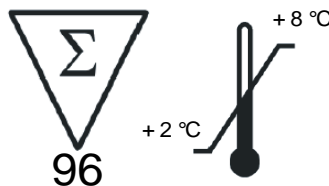


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1. INTENDED USE

The BAFF, Soluble (human) Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) Kit is intended for the quantitative determination of soluble (human) BAFF in serum and cell culture supernatant. It is for *in vitro* diagnostic use only.

2. INTRODUCTION

BAFF (B cell activating factor belonging to the TNF family, also known as BLyS or TALL1) is a cytokine expressed predominantly by cells of the immune system such as neutrophils, monocytes, macrophages, dendritic cells, follicular dendritic cells, activated T cells and some malignant B cells. BAFF binds three distinct receptors (BAFFR, TACI and BCMA) predominantly expressed on B cells, although activated T cells also express BAFFR. BAFF is a master regulator of peripheral B cell survival, and also acts in processes such as immunoglobulin isotype switch and B cell co-stimulation. Beside its major role in B cell biology, BAFF co-stimulates activated T cells. Deregulated expression of this membrane-bound protein, which can readily be released in a soluble form by proteolytic cleavage, leads to autoimmune disorders in mice. In the human, elevated levels of soluble BAFF have been detected in the serum of patients with various autoimmune diseases, such as rheumatoid arthritis (RA), Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE). BAFF levels are also elevated in patients with multiple myeloma and B-cell chronic lymphoid leukemia (B-CCL).

3. MATERIAL SUPPLIED

Catalogue No	Content	Kit Components	Quantity
K 9410MTP	PLATE	One holder with precoated strips	12 x 8 wells
K 9410WB	WASHBUF	ELISA wash buffer concentrate 10x	100 ml
K 9410AP	ASYBUF	Assay buffer, ready-to-use	16 ml
K 9410	CONJBUF	Conjugate dilution buffer, ready-to-use	25 ml
K 9410	STDBUF	Standard dilution buffer, ready-to-use	16 ml
K 9410ST	STD	BAFF Standards, lyophilized (for range see specification or label)	2 x 8 vials
K 9410	AB	Detection antibody, monoclonal anti BAFF antibody, concentrate	250 μ l
K 9410K	CONJ	Conjugate, peroxidase-labeled, concentrate	200 μ l
K 9410TMB	SUB	TMB substrate (Tetramethylbenzidine), ready-to-use	2 x 15 ml
K 9410AC	STOP	ELISA stop solution, ready-to-use	7 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled (aqua bidest.) and sterile water
- Laboratory balance
- Precision pipettors calibrated and tips to deliver 5-1000 μ l
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 or 405 nm (reference wave length 620 or 690 nm)

5. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted with aqua bidest. **1:10** before use (100 ml concentrate + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or at 37°C before dilution of the buffer solutions. The **buffer concentrate** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month**.
- The lyophilized **standards** (STD) are stable at 2-8°C until the expiry date stated on the label. The standards must be reconstituted with **500 µl standard dilution buffer** (STDDBUF). Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted standards are **not stable**.
- The **detection antibody** (AB) must be diluted **1:100** in wash buffer (WASHBUF) (100 µl AB + 10 ml WASHBUF). The antibody is stable at **2-8 °C** until expiry date stated on the label. **Diluted antibody solution is not stable and can not be stored.**
- The **conjugate** (CONJ) must be diluted **1:150** in conjugate dilution buffer (CONJBUF) (65 µl CONJ + 10 ml CONJBUF). The undiluted conjugate is stable at **2-8 °C** until expiry date stated on the label. **Diluted conjugate is not stable and can not be stored.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C**.

6. SAMPLE PREPARATION

Serum and cell culture supernatant

Centrifugation of serum or cell culture supernatant is recommended before apply to kit.

7. ASSAY PROCEDURE

Principle of the test

This assay is a sandwich (ELISA) for the direct measurement of human BAFF in serum and cell culture supernatants.

Standards, controls and samples containing human BAFF are added to wells of microplate coated with a high affinity monoclonal anti-human BAFF antibody. During the first incubation period, the antibody immobilized on the wall of the microtiter wells captures BAFF in the patient samples or in the cell culture supernatants. After washing away the unbound components from samples, a detection antibody (monoclonal anti-BAFF antibody) is added to each well. During the incubation step, the detection antibody is bound to the captured BAFF. A peroxidase-conjugated antibody is then added to each microtiter well and a "sandwich" of capture antibody - human BAFF - detection antibody - Peroxidase conjugate is formed. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally, an acidic stop solution is added to terminate the reaction. The intensity of the yellow color is directly proportional to the BAFF concentration of sample. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated; using the values obtained from standard. BAFF present in the patient samples or cell culture supernatants, is determined directly from this curve.

Test procedure

1. Bring all reagents and samples to room temperature (18-26 °C) and mix well
2. Mark the positions of STD /SAMPLE (Standards/Sample) in duplicate on a protocol sheet
3. Take as many microtiter strips as needed from kit. Store unused strips covered at 2-8° C. Strips are stable until expiry date stated on the label
4. Wash each well 5 times by dispensing 300 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper
5. Add 50 µl of STD/SAMPLE (Standard/Sample; see section 5) in duplicate into respective well
6. Add 150 µl ASYBUF (Assay buffer) into each well
7. Cover the plate tightly and incubate for 2 hours at room temperature (18-26°C) on a horizontal mixer
8. Aspirate the contents of each well. Wash 5 times by dispensing 300 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
9. Add 200 µl AB (detection antibody) (1:100, see section 5) into each well

10. Cover plate tightly and incubate for 1,5 hours at room temperature (18-26°C) on a horizontal mixer
11. Aspirate the contents of each well. Wash 5 times by dispensing 300 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
12. Add 200 µl CONJ (conjugate) (1:150, see section 5) into each well
13. Cover plate tightly and incubate for 2 hours at room temperature (18-26°C) on a horizontal mixer
14. Aspirate the contents of each well. Wash 5 times by dispensing 300 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper to remove excess solution
15. Add 200 µl of SUB (substrate) into each well
16. Incubate for 10 - 20 minutes at room temperature (18-26°C) in the dark*
17. Add 50 µl of STOP (stop solution) into each well, mix thoroughly

18. Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference

*The intensity of the color change is temperature sensitive. We recommend to observe the procedure of the color change and to stop the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use the "4-Parameter-algorithm".

1. 4-parameter-algorithm

It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

2. Point-to-point-calculation

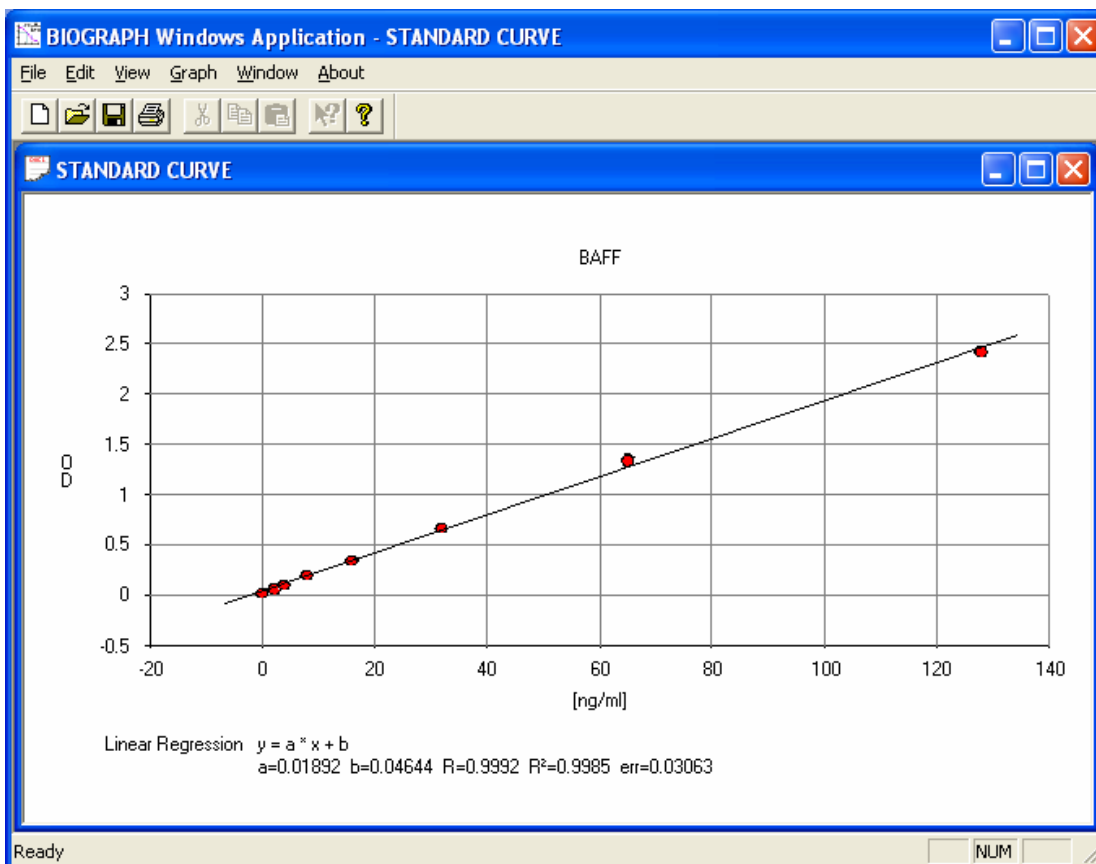
We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. Spline-algorithm

We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.01).

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Typical calibration curve



STD	OD1	OD2	mean OD	CV (%)	Conc. [ng/ml]
1	0.027	0.023	0.025	11.3	0
2	0.068	0.060	0.064	8.8	2
3	0.112	0.107	0.110	3.2	4
4	0.204	0.195	0.200	3.2	8
5	0.350	0.356	0.353	1.2	16
6	0.673	0.672	0.673	0.1	32
7	1.372	1.314	1.343	3.1	64
8	2.435	2.422	2.429	0.4	128

The data is for demonstration only and cannot be used for the evaluation of test results.

9. LIMITATIONS

Cell culture supernatant or serum with BAFF levels greater than the highest standard value, should be diluted with the corresponding cell culture medium or STDBUF, respectively and re-assayed.

10. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

11. PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity was set as $B_0 + 3SD$. The zero-standard was measured 20 times.

Sample	BAFF mean value [OD]	Standard variation (SD)	Detection limit [ng/ml]
1	0.030	0.006	0.95

12. PRECAUTIONS

- For *in vitro* diagnostic use only.
- Quality control guidelines should be observed.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- Guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from wrong use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG or Apotech Corporation along with a written complaint.

15. REFERENCES

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This assay was developed by
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