Quantum Blue®
Adalimumab

Quantitative
Lateral Flow Assay

For research use only.
Not for use in diagnostic procedures.

LF-TLAD25-U 25 tests

LF-TLAD10-U 10 tests

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Version A1

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ENGLISH

INTENDED USE

BÜHLMANN Quantum Blue® Adalimumab is a lateral flow immunoassay for the quantitative determination of trough levels of adalimumab in serum samples.

For research use only. Not intended for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The test is designed for the selective measurement of adalimumab by a sandwich immunoassay. Recombinant tumor necrosis factor alpha (TNFα) is conjugated to gold colloids. On the test cassette the gold conjugate is released from a pad into the reaction system as the sample is applied. Adalimumab present in the sample will bind to the gold conjugate. A monoclonal antibody, highly specific for adalimumab, is immobilized on the analytical membrane and will capture the complex of gold conjugate and the adalimumab analyte, resulting in a coloring of the Test Line (T). The remaining free TNFα-gold conjugate will bind to the Control Line (C). The signal intensities of the Test Line (T) and the Control Line (C) are measured quantitatively by the Quantum Blue® Reader.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity		Code	Comments
	LF-TLAD25	LF-TLAD10		
Test Cassette	25 pieces	10 pieces	B-LFTLAD-TC	vacuum-sealed in a foil bag pouch
Chase Buffer	1 bottle 10 mL	1 bottle 10 mL	B-LFTLAD-CB	Ready to use
Controls Low* / High*	2 vials, 0.5 mL	2 vials, 0.5 mL	B-LFTLAD-CONSET	Ready to use
RFID Chip Card	1 piece	1 piece	B-LFTLAD-RCC	**White** plastic card
RFID Chip Card	1 piece	1 piece	B-LFTLAD-RCC15	**Green** plastic card

Table 1

* The controls contain lot specific amounts of adalimumab. Refer to the additional QC data sheet for actual concentrations.

CHECK YOUR TEST KIT

BÜHLMANN products have been manufactured with the greatest of care and all possible efforts have been taken to ensure completeness of this test kit and its performance. Nevertheless, we advise you to verify your test kit for the condition of the test cassette and its pouch based on the following criteria:

- Expiration date
- The fault-free condition of the pouch (e.g. absence of any perforation that could be caused by improper handling).
- The fault-free condition of the test cassette (e.g. absence of scratches on the analytical membrane).

Should one of the test cassettes not fulfil the criteria mentioned above, please use another test cassette.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened reagents	
Store at 2-8 °C. Do not use the reagents beyond the expiration date printed on the labels.	
Opened reagents	
Test Cassette	Test cassettes removed from the foil pouch must be used within 4 hours.
Chase Buffer	Store for up to 6 months at 2-8 °C after opening.
Controls Low / High	Store for up to 6 months at 2-8 °C after opening.

Table 2

MATERIALS REQUIRED BUT NOT PROVIDED

- Vortex mixer
- Timer (optional)
- Precision pipettes with disposable tips: 10-100 µL and 100-1000 µL
- Eppendorf tubes (or equivalent) for dilution of serum samples
- Quantum Blue® Reader available from BÜHLMANN (order code: BI-POCTR-ABS)
- Gloves and laboratory coat

PRECAUTIONS

Safety precautions

- None of the reagents of this test contain components of human origin.
- Specimens should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practice (GLP) using appropriate precautions.
- Reagents: Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, irritation can occur.
- Unused solution should be disposed according to local state and federal regulations.

Technical precautions

Kit components

- The test must be performed at room temperature (16-28 °C).
- All reagents, including test cassettes in foil pouches, and test samples must be equilibrated to room temperature before starting the assay.
- Before performing the test, remove the test cassette from the foil pouch. Allow the test cassette to equilibrate in the laboratory environment for at least 2 minutes. Test cassettes removed from the foil pouch must be used within 4 hours.
- Mix well (e.g. vortex) the reagents before use.
- Components must not be used after the expiration date printed on the labels.
- Do not mix different lots of reagents.
- Do not disassemble the test cassettes.
- Test cassettes cannot be re-used.

- Handle the test cassettes with care. Do not contaminate the sample loading port or read-out window via skin contact, other liquids, etc. (figure 1D).
- Ensure a flat, horizontal position of the test cassette while performing the assay.

Test Procedure

- Read carefully the instructions prior to carrying out the test. Test performance will be adversely affected, if reagents are incorrectly diluted, handled or stored under conditions other than those as detailed in this instruction for use.
- The Quantum Blue® Reader must be switched on and programmed for the Quantum Blue® Adalimumab assay: Load the test method using the RFID chip card (B-LFTLAD-RCC or B-LFTLAD-RCC15) before starting the assay (see Quantum Blue® Reader Manual).
- Use the RFID chip card in order to change lot-specific test parameters.
- Samples that are not properly handled may cause inaccurate results.
- Diluted samples should be stored at 2-8 °C and measured within 24 hours. The diluted samples cannot be stored for a longer period.
- Samples above 35 µg/mL (up to 500 µg/mL) may be additionally diluted 1:20 in chase buffer (1:400, in total) to obtain results within the measuring range of the test.

SPECIMEN COLLECTION AND STORAGE

Collect blood into plain venipuncture tubes without any additives avoiding hemolysis and let the serum clot at room temperature for at least 20 and for up to 60 minutes. Centrifuge at room temperature at ~2000 x g for 15 minutes. Decant the serum.
Serum samples can be stored refrigerated at 2-8 °C for up to 14 days. For longer storage, keep serum samples at ≤-20 ºC. These samples are stable for at least 3 months at ≤-20 ºC.

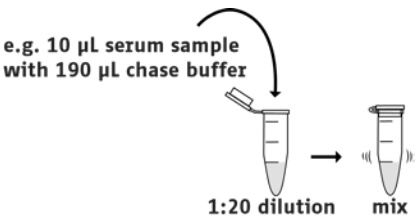
ASSAY PROCEDURE

For the assay use only reagents equilibrated to room temperature (16-28 °C). The test cassette must be removed from the foil pouch prior to assay start.

The assay procedure consists of two steps:

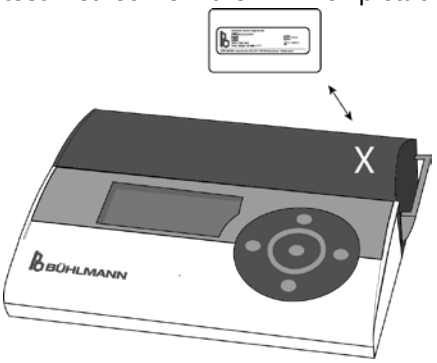
1. Dilution of serum samples with chase buffer

Prior to measurement dilute the serum sample 1:20 with chase buffer (B-LFTLAD-CB) (e.g. mix 10 µL serum sample with 190 µL chase buffer) in a test tube and mix it by vortexing, pipetting or shaking.



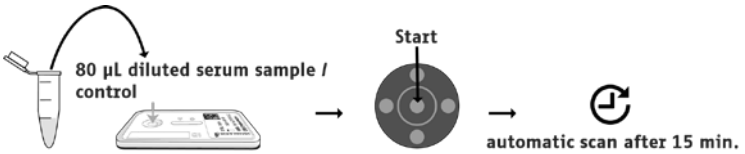
2. Lateral flow assay procedure and readout

Two alternative methods can be loaded from the respective RFID chip card: TLAD_0 or TLAD_15. Select one of the RFID chip cards before starting the experiments. Load the test method from the RFID chip card.



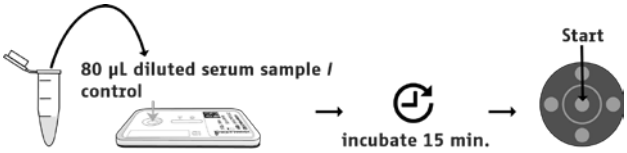
2.1. Method <TLAD_15> with internal timer

- Use the green plastic card.
- Insert the test cassette into the test cassette holder of the Quantum Blue® Reader.
- Add 80 µL of the diluted serum sample onto the sample loading port of the test cassette (figure 1D).
- Close the test cassette holder and start the measurement by pressing the start button.
- The scan starts automatically after 15 minutes (900 seconds).
- For low / high controls: Repeat step 2.1 using 80 µL of control instead of diluted serum.



2.2. Method <TLAD_0> without internal timer

- Use the white plastic card.
- Add 80 µL of the diluted serum sample onto the sample loading port of the test cassette (figure 1D).
- Incubate for 15 ± 1 minutes (set a timer manually).
- Insert the test cassette into the test cassette holder of the Quantum Blue® Reader.
- Scan the test cassette with the Quantum Blue® Reader by pressing the start button immediately.
- For low / high controls: Repeat step 2.2 using 80 µL of control instead of diluted serum.



Remark: Please refer to the Quantum Blue® Reader Manual to learn about the basic functions and how to initialize and operate the Quantum Blue® Reader, especially how to select test methods, and how to load lot specific parameters from the RFID chip card in order to get the samples measured. Ensure the correct insertion of the test cassette into the Quantum Blue® Reader, with the read-out window first (figure 1D).

QUALITY CONTROL

- If the performance of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: *i)* pipetting, temperature controlling and timing *ii)* expiration dates of reagents and *iii)* storage and incubation conditions.
- Result of the self-test of the Quantum Blue® Reader performed at the startup of the instrument has to be valid.

STANDARDIZATION

- The Quantum Blue® Adalimumab is calibrated with the original biological using a CE -certified quantitative Immunoglobulin G assay. The standard curve parameters are indicated in the enclosed QC data sheet.
- The Quantum Blue® Reader uses a lot-specific calibration curve to calculate the adalimumab concentration. The measuring range is between 1.3 and 35.0 µg/mL.

VALIDATION OF RESULTS

- For a valid test result, the Control Line (C) must be visible in all cases (see figure 1A and figure 1B). It is used as a functional test control only and cannot be used for the interpretation of the Test Line (T). If the Test Line (T) is not detectable after 15 minutes of incubation time (figure 1A), the concentration of adalimumab present in the serum sample is below the detection limit. If a Test Line (T) is detectable after 15 minutes of incubation time (figure 1B), the concentration present in the serum sample is calculated by the Quantum Blue® Reader.
- If only the Test Line (T) is detectable after 15 minutes of incubation time (figure 1C), the test result is invalid and the Quantum Blue® Adalimumab assay has to be repeated using another test cassette.
- If neither the Control Line (C) nor the Test Line (T) are detectable after 15 minutes of incubation time (figure 1D), the test result is invalid and the Quantum Blue® Adalimumab assay has to be repeated using another test cassette.
- As the Quantum Blue® Reader allows a quantitative evaluation of the Test (T) and Control (C) Line, an additional validity check of the Control Line (C) is undertaken. If the signal intensity of the Control Line (C) is below a threshold after 15 minutes of incubation time, the test result is also invalid and the Quantum Blue® Adalimumab assay has to be repeated using another test cassette.

LIMITATIONS

- The reagents supplied with this kit are optimized to measure trough levels of adalimumab in diluted serum samples.

PERFORMANCE CHARACTERISTICS

Method comparison

Bias at 5 µg/mL: 0.3 % (95 % CI: -8.1-6.8 %)

Bias at 12 µg/mL: 13.8 % (95 % CI: 7.9-21.7 %)

The method comparison study was performed according to the CLSI guideline EP09-A3. One hundred and thirty (130) samples were measured in triplicate with the Quantum Blue® Adalimumab test, resulting in 390 values, and with a commercially available adalimumab ELISA test. Measurements were performed over four days using two Quantum Blue® Adalimumab test cassette lots. The results are summarized in figure 2.

Recovery: 80-90 %

Six specimens were spiked with 5.44 µg/mL adalimumab in serum-based calibrator material. “Base” samples were spiked with the corresponding amount of filtered, pooled normal human serum. “Base” and “base + spike” samples were measured in ten replicates with one reagent lot. The results are shown in table 3.

Repeatability: 16.6-28.6 % CV

Within-laboratory precision: 19.1-29.9 %CV

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3. Four, pooled serum samples with adalimumab concentrations covering the measuring range of the assay were tested over 20 days, in two independent runs with two replicates per run. The results are summarized in table 4.

Reproducibility: 25.6-26.1 % CV

Reproducibility was established according to the CLSI guideline EP05-A3 by performing measurements on three different Quantum Blue® Reader instruments with three different test cassette lots. Four, pooled serum samples with adalimumab concentrations covering the measuring range of the assay were tested over five days, in one independent run with five replicates per run. Each Quantum Blue® Reader was operated by a different operator using a different test cassette lot. The results are summarized in table 5.

Limit of Blank (LoB): 0.2 µg/mL

The LoB was established according to the CLSI guideline EP17-A2 with four, negative serum samples. The samples were measured over three days in five replicates each day to produce 60 blank values. The study was performed with two test cassette lots. The LoB was calculated using non-parametric analysis.

Limit of Detection (LoD): 0.8 µg/mL

The LoD was established according to the CLSI guideline EP17-A2 with four adalimumab samples with concentrations of 0.3, 0.4, 0.5 and 0.9 µg/mL adalimumab. The samples were measured over three days in five replicates each day to produce 60 values. The study was performed with two test cassette lots. The LoD was calculated using parametric analysis.

Lower Limit of Quantitation (LLoQ): 1.3 µg/mL

Upper Limit of Quantitation (ULoQ): 35.0 µg/mL

The LLoQ was established according to the CLSI guideline EP17-A2 with six samples; the ULoQ with five samples. The samples were measured over at least three days, in five replicates each day to produce 90 (LLoQ) and 75 (ULoQ) values. The study was performed with two test cassette lots. A precision profile was generated for the samples and the LoQ was determined as the intersection of the profile with the <30 % CV acceptance criterion.

Linear range: 1.0-35.0 µg/mL

The linear range of the Quantum Blue® Adalimumab test was determined according to the CLSI guideline EP06-A. Two sample pools, low and high, were blended to obtain a total of 15 concentration levels covering and exceeding the expected measuring range. The blends were assayed in ten replicates on two test cassette lots. The linear range was defined as the concentration interval in which coefficients of the second and third order non-linear fits were determined as not significant. The results are summarized in figure 3.

Samples with elevated adalimumab levels (up to 500 µg/mL) may be additionally diluted 1:20 in chase buffer (1:400, in total) to obtain linear results within the measuring range of the assay. A series of samples with adalimumab concentrations in the range of 7 to 800 µg/mL was generated by blending a high, contrived sample with negative serum. The samples were diluted twice 1:20 in chase buffer and measured in five replicates with the Quantum Blue® Adalimumab test. A linear range was determined for adalimumab levels between 7 and 502 µg/mL.

High dose hook effect

No high dose hook effect was observed for samples with adalimumab concentrations of up to 787 µg/mL. The presence of a high dose hook effect was tested on three independent test cassette lots.

INTERFERING SUBSTANCES

The susceptibility of the Quantum Blue® Adalimumab test to interfering substances was assessed according to the CLSI-approved guideline EP7-A2. Interfering substances were tested at concentrations three-fold higher than those reported or expected in samples or at concentration levels recommended by the CLSI guideline EP07-A2. Bias exceeding 30 % was considered interference.

Within-class switch / TNFα blocker

No interference was detected with the following substances at the listed concentrations: infliximab (Remicade®, 10 µg/mL), and golimumab (Simponi®, 10 µg/mL). Interference was detected with etanercept (Enbrel®) with the 95 % confidence interval of the interference trend exceeding acceptable bias at 2.7 µg/mL. Samples from people switching from certolizumab (Cimzia®) should not be directly tested using the Quantum Blue® Adalimumab test. Allow certolizumab (Cimzia®) trough levels to fall at least below 2.9 µg/mL.

Serum indices

No interference was detected with the following substances, up to the listed concentrations: Triglycerides (Intralipid® 1320 mg/dL; equivalent to 37 mmol/L triglyceride), conjugated bilirubin (342 µmol/L; 29 mg/dL), unconjugated bilirubin (342 µmol/L; 20 mg/dL), hemoglobin (200 mg/dL), TNFα (5.0 ng/mL) and rheumatoid factors (823 IU/mL).

Immunosuppressive co-medication

No interference was detected with the following substances, up to the listed concentrations: azathioprine (60 µmol/L, 3 µg/mL), 6-mercaptopurine (37 µmol/L, 2 µg/mL), and methotrexate (1363 µmol/L, 68 µg/mL).

APPENDIX I

TABLES AND FIGURES

Test results

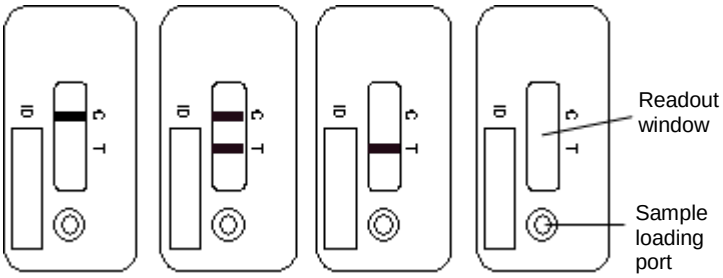


Figure 1A Figure 1B Figure 1C Figure 1D

Method comparison

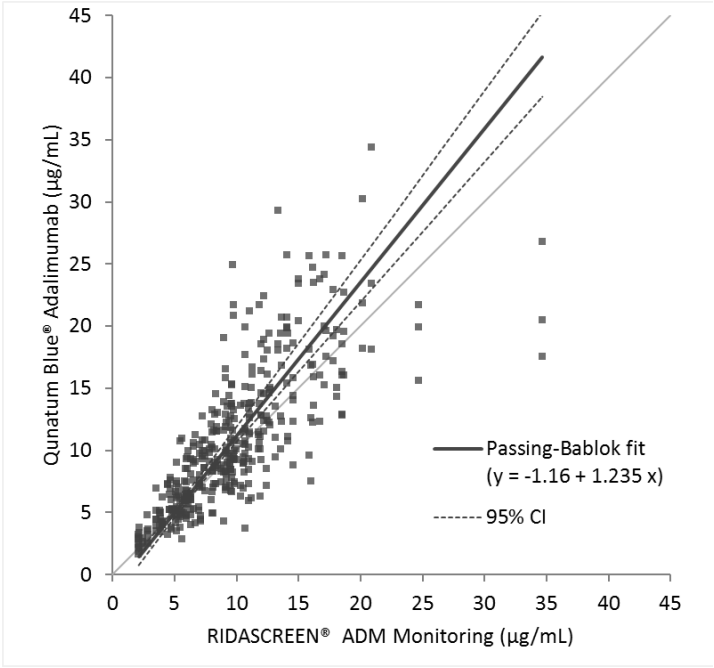


Figure 2

Recovery

Sample	Base [µg/mL]	Spike [µg/mL]	Expected Base + Spike [µg/mL]	Observed Base + Spike [µg/mL]	Recovery [%]
S1	2.6	5.44	8	6.7	83
S2	4.6	5.44	10.1	9	89
S3	5.2	5.44	10.7	8.6	80
S4	8.1	5.44	13.5	11.1	82
S5	8.5	5.44	13.9	12.5	90
S6	12.2	5.44	17.6	15.2	86

Table 3

Repeatability / within-laboratory precision

Mean ADA Conc. [µg/mL]	Repeatability CV [%]	Between-run precision CV [%]	Between-day precision CV [%]	Within-lab precision CV [%]
2.03	18.7	3.4	1.6	19.1
6.63	16.6	12.6	0.0	20.9
9.40	17.8	7.3	1.1	19.3
22.70	28.6	3.6	8.0	29.9

Table 4

Reproducibility

Mean ADA Conc. [µg/mL]	Repeatability CV [%]	Between-day precision CV [%]	Between-lot/ instrument /operator precision CV [%]	Within-lab precision CV [%]
2.46	18.7	3.4	1.6	19.1
7.62	16.6	12.6	0.0	20.9
9.40	17.8	7.3	1.1	19.3
22.70	28.6	3.6	8.0	29.9

Table 5

Linearity

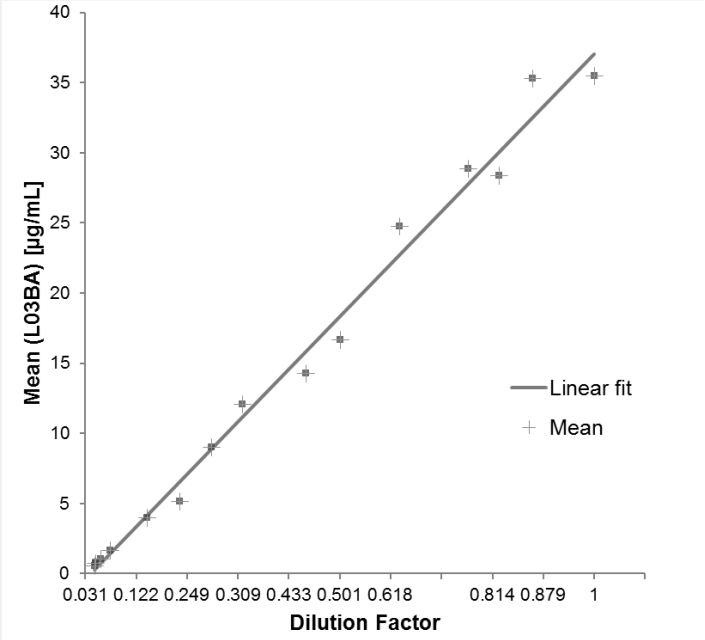


Figure 3

APPENDIX II

NOTES

APPENDIX III

SYMBOLS

Symbol	Explanation
	Use By
	Catalogue Number
	Batch Code
	Contains sufficient for <n> tests
	Consult Instructions for Use
	Temperature Limitation

Symbol	Explanation
	Test Cassette
	Chase Buffer
	Control Low
	Control High
	RFID Chip Card