Quantum Blue®
hsCRP

Quantitative
Lateral Flow Assay

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

LF-CRP25-U 25 tests

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Version A1

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ENGLISH

INTENDED USE

Quantum Blue® hsCRP is a lateral flow immunoassay for the quantitative determination of C-Reactive Protein (CRP) in human serum samples. Quantum Blue® hsCRP is combined with the Quantum Blue® Reader.

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PRINCIPLE OF THE ASSAY

The test is designed for the selective measurement of CRP antigen by a sandwich immunoassay. A monoclonal detection antibody, specific for CRP, 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. CRP present in the sample will bind to the gold conjugate. A second monoclonal capture antibody, highly specific for CRP, is immobilized on the analytical membrane and will capture the complex of gold conjugate and the CRP antigen, resulting in a coloring of the test line (T). The remaining free gold conjugate will bind to the antibody of 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
Test Cassette	25 pieces	B-LFCRP-TC	Vacuum sealed in a foil bag pouch
Chase Buffer	1 bottle 10 mL	B-LFCRP-CB	Ready to use
RFID Chip Card	1 piece	B-LFCRP-RCC	**White** plastic card
RFID Chip Card	1 piece	B-LFCRP-RCC15	**Green** plastic card

Table 1

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).

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.

Table 2

MATERIALS REQUIRED BUT NOT PROVIDED

- Vortex mixer
- Timer
- Precision pipettes with disposable tips: 1-10 µL, 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.
- Serum samples 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 (18-26 °C)
- All reagents, including test cassettes in foil pouches, and serum samples must be equilibrated to room temperature before starting the assay.
- 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 the instructions carefully 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® hsCRP assay before starting the assay (see Quantum Blue® Reader manual).
- Use the RFID chip card in order to change lot-specific test parameters.
- Serum samples that are not handled properly may cause inaccurate results.
- Diluted serum samples may be stored at laboratory conditions[1] for up to 24 hours before being tested. The diluted serum samples cannot be stored for a longer period.

SPECIMEN COLLECTION AND STORAGE

Collect blood into plain venipuncture tubes without any additives and avoid hemolysis. Perform serum preparation according to manufacturer's instructions. Decant the serum.
Undiluted serum samples can be stored refrigerated at 2-8 °C for up to 14 days. For longer storage, keep undiluted serum samples at ≤-20 ºC. The samples are stable for up to 6 months at ≤-20 ºC.

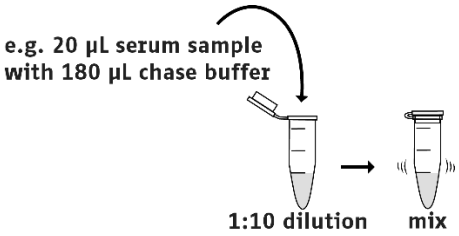
ASSAY PROCEDURE

For the assay use only reagents equilibrated to room temperature (18-26 °C). The test cassette must be removed from the foil pouch prior to assay start.

The assay procedure consists of two steps:

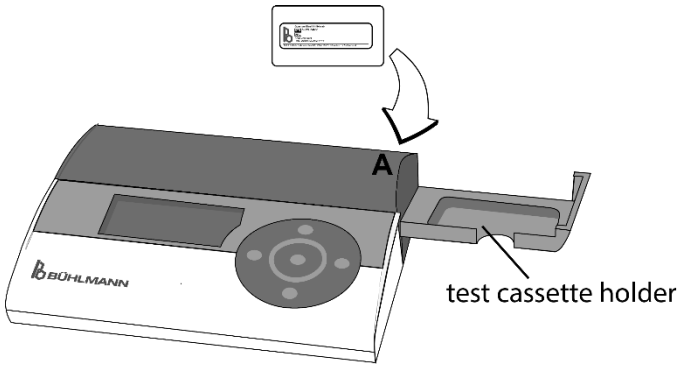
1. Dilution of serum samples

Prior to measurement, dilute the serum sample 1:10 with chase buffer (B-LFCRP-CB) (e.g. 20 µL serum sample and 180 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: CRP_0 (without internal timer) or CRP_15 (with internal timer). Select one of the RFID chip cards before starting the experiments. Load the test method from the RFID chip card by holding it for a few seconds at position "A" on the Quantum Blue® Reader.

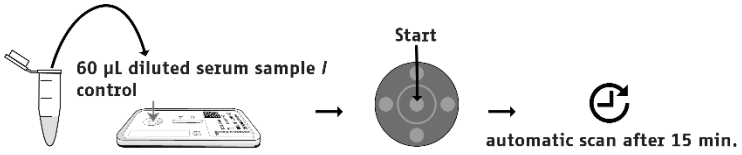


2.1 Method <CRP_0> without internal timer

- Use the white RFID chip card
- Add 60 µL of 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.
- If using internal control samples; Repeat step 2.1 using 60 µL of control instead of diluted sample.

2.2 Method <CRP_15> with internal timer

- Use the green RFID chip card
- Insert the test cassette into the test cassette holder of the Quantum Blue® Reader.
- Add 60 µL of 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.
- If using internal control samples; Repeat step 2.2 using 60 µL of control instead of diluted sample.



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 on the Quantum Blue® Reader. Ensure the correct insertion of the test cassette into the Quantum Blue® Reader, with the read-out window first (figure 1D).

[1] Diluted sample stability was tested at 21°C.

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 iii) storage and incubation conditions.

Result of the self-test of the Quantum Blue® Reader performed at startup has to be valid.

QC controls

It is recommended to use internal control samples. The use of internal control samples is advised to assure the validity of results. BÜHLMANN recommends the use of CRPL3 Multi level 1 (Roche, 05117003 190, target values established for Roche cobas® c501) controls.

STANDARZIDATION

- Calibrator values of the standard curve are assigned according to a value transfer protocol. The standard curve parameters are indicated in the enclosed QC data sheet.
- The calibrator material comprises human CRP in a CRP depleted serum matrix and is standardized against certified reference material ERM-DA474/IFCC.
- The Quantum Blue® Reader uses a lot-specific calibration curve to calculate the CRP concentration. The analytical measuring interval is between 1.0 and 20.0 mg/L.
- Serum samples with elevated CRP levels (up to 724 mg/L) may be additionally diluted 1:20 in chase buffer (1:400, in total) to obtain linear results within the measuring range of the assay.

VALIDATION OF RESULTS

- For a valid test result, the control line (C) must be visible in all cases (see figure 1A and 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 tine (T) is not detectable after 15 minutes of incubation time (figure 1A), the concentration of CRP present in the sample is below the detection limit. If a test line (T) is detectable after 15 minutes of incubation time (figure 1B), the CRP concentration present in the 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® hsCRP assay has to be repeated using another test cassette.
- If neither the control line (C) nor the test line (T) is detectable after 15 minutes of incubation time (figure 1D), the test result is invalid and the Quantum Blue® hsCRP assay has to be repeated using another test cassette.
- The Quantum Blue® Reader performs an additional validity check of the control line (C). If the signal intensity of the control line (C) is below a specific preconfigured threshold after 15 minutes of incubation time, the test result is invalid and the Quantum Blue® hsCRP assay has to be repeated using another test cassette.

PERFORMANCE CHARACTERISITCS

Method comparison
Bias at 5.0 mg/L: 3.7% (95% CI: -0.3% - 6.3%)

The method comparison study was performed according to the CLSI guideline EP09-A3. Ninety-five (95) samples were measured in triplicates with the Quantum Blue® hsCRP test, resulting in 285 values, and compared to the Roche C-Reactive Protein Gen.3 assay. Measurements were performed over six days using three different Quantum Blue® hsCRP test cassette lots. The results are summarized in figure 2.

Recovery: 92.3-108.4%

Five serum samples covering the measuring range of the assay were spiked with 5.0 mg/L CRP in serum-based calibrator material. “Base” samples were spiked with the corresponding amount of CRP depleted serum. “Base” and “base + spike” samples were measured in nine replicates with one reagent lot. The results are shown in table 3.

Repeatability: 10.3-14.4% CV
Within-laboratory precision: 11.4-17.3% CV

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3. Four pooled serum samples with CRP 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.

Intermediate precision: 11.3-18.6% CV

Intermediate precision was established according to the CLSI guideline EP05-A3 by performing measurements on two different Quantum Blue® Readers with a different test cassette lot on each reader. For each reader/test-cassette lot combination four pooled serum samples with CRP concentration 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 5.

Limit of Blank (LoB): 0.15 mg/L

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 LoB was calculated using non-parametric analysis. The study was performed with two test cassette lots, taking the higher estimate obtained with one lot as the claimed LoB value.

Limit of Detection (LoD): 0.37 mg/L

The LoD was established according to the CLSI guideline EP17-A2 with four serum samples with concentrations of 0.3, 0.38, 0.61, and 0.72 mg/L of CRP. The samples were measured over three days in five replicates each day to produce 60 single values. The LoD was calculated using parametric analysis. The study was performed with two test cassette lots, taking the higher estimate obtained with one lot as the claimed LoD value.

Lower Limit of Quantitation (LLoQ): 0.39 mg/L
Upper Limit of Quantitation (ULoQ): 21.1 mg/L

The LLoQ was established according to the CLSI guideline EP17-A2 with seven serum samples; the ULoQ with five serum samples. The samples were measured over three

(LLoQ) and five (ULoQ) days, in five replicates each day to produce 105 and 75 values, respectively.
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. The study was performed with two test cassette lots, taking the higher and lower estimate obtained with one lot as the claimed LLoQ and ULoQ value, respectively.

Linear range: 0.7-30.3 mg/L

The linear range of the Quantum Blue® hsCRP test was determined according to the CLSI guideline EP06-A. Two serum sample pools, low and high, were blended to produce a total of 17 concentration levels covering and exceeding the expected measuring range. The blends were assayed in nine replicates on two test cassette lots. The linear range was defined as the interval of concentration levels in which coefficients of the second and third order non-linear fits were determined as not significant. Results for one test cassette lot are shown in figure 3.
Serum samples with elevated CRP levels (up to 724 mg/L) may be additionally diluted 1:20 in chase buffer (1:400, in total) to obtain results within the linear measuring range of the assay. A series of samples with CRP concentrations in the range of <1.0 to >1000 mg/L was generated by blending a high, contrived sample with CRP depleted serum, i.e. a negative matrix. Samples were diluted twice 1:20 in chase buffer and measured in five replicates with the Quantum Blue® hsCRP test on two test cassette lots. A linear range was determined for CRP levels between 4.8 and 724 mg/L.

High dose hook effect

No high dose hook effect limitations were observed for serum samples with CRP concentrations of up to 1000 mg/L. The presence of a high dose hook effect was tested on two independent test cassette lots.

INTERFERING SUBSTANCES

The susceptibility of the Quantum Blue® hsCRP test to interfering substances was assessed according to the CLSI-approved guideline EP7-A2. Bias exceeding 30% was considered interference.

TNFα blocker

TNFα blockers were tested at concentrations exceeding lowest, recommended drug trough levels by three-fold. No interference was detected with the following substances at the listed concentrations: Infliximab (Remicade®, 10 µg/mL), Adalimumab (Humira®, 15 µg/mL) and Etanercept (Enbrel®, 10 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 g/L) and rheumatoid factors (662 IU/mL).

Commonly used medications

No interference was detected with the following substances, up to the listed concentrations: Leflunomid (24.4 µmol/L, 6.6 µg/mL), methotrexate (1363 µmol/L, 68 µg/mL), paracetamol (Perfalgan® 595 µmol/L, 90 µg/mL) and diclofenac (Voltaren® 4.7 µmol/L, 1.5 µg/mL).

APPENDIX I

TABLES AND FIGURES

Validation of results

Test results

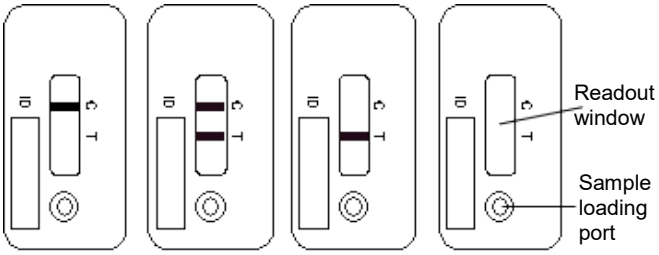


Figure 1A Figure 1B Figure 1C Figure 1D

Method comparison

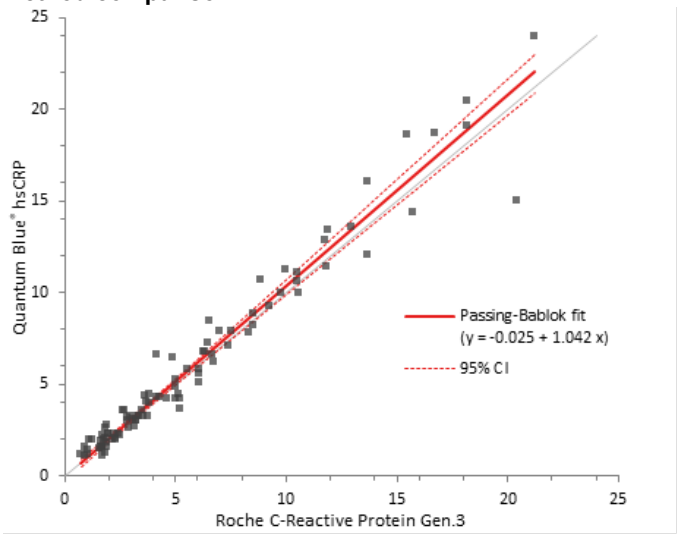


Figure 2

Recovery

Sam-ple	Base [mg/L]	Spike [mg/L]	Expected Base + Spike [mg/L]	Observed Base + Spike [mg/L]	Recovery [%]
6610	1.3	5.0	6.3	6.0	95.4
6573	2.3	5.0	7.3	6.7	92.3
6604	4.2	5.0	9.2	10.0	108.4
6582	6.0	5.0	11.0	11.3	102.2
6598	6.7	5.0	11.7	12.4	106.0

Table 3

Repeatability/ Within-laboratory precision

Mean CRP conc.	Within-run (repeatability)		Between-run		Between-day		Within-Laboratory (total)	
[mg/L]	SD	CV [%]	SD	CV [%]	SD	CV [%]	SD	CV [%]
1.79	0.24	13.3	0.0	0.0	0.10	5.7	0.26	14.5
2.83	0.38	13.4	0.0	0.0	0.17	5.8	0.41	14.6
5.31	0.55	10.3	0.26	4.9	0.00	0.0	0.60	11.4
10.00	1.44	14.4	0.0	0.0	0.96	9.6	1.73	17.3

Table 4

Intermediate precision

Mean CRP conc.	Within-run (repeatability)		Between-run		Between-day		Between-lot/ instrument precision		Total precision	
[µg/mL]	SD	CV [%]	SD	CV [%]	SD	CV [%]	SD	CV [%]	SD	CV [%]
1.75	0.23	13.3	0.0	0.0	0.06	3.7	0.04	2.5	0.25	14.0
2.76	0.39	14.0	0.0	0.0	0.11	4.0	0.00	0.0	0.40	14.5
5.20	0.57	10.9	0.12	2.4	0.07	1.4	0.00	0.0	0.59	11.3
9.40	1.38	14.7	0.0	0.0	0.92	9.8	0.57	6.0	1.75	18.6
9.36	1.33	14.2	0.0	0.0	0.87	9.3	0.50	5.3	1.67	17.8

Table 5

Linearity Plot

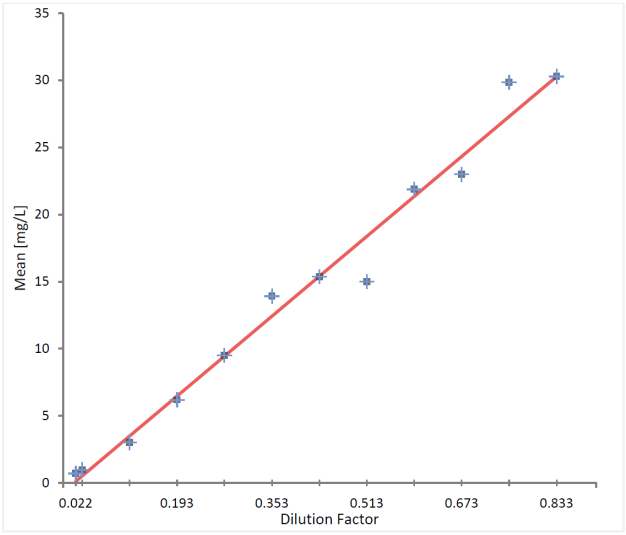


Figure 3

APPENDIX II

NOTES

APPENDIX III

SYMBOLS

Symbol	Explanation	Symbol	Explanation
	Use by		Temperature limitation
	Catalogue number		Manufacturer
	Batch code		Test Cassette
	Contains sufficient for <n> tests		Chase Buffer
	Consult instructions for use		RFID Chip Card