BÜHLMANN sCAL® turbo

Serum calprotectin turbidimetric assay

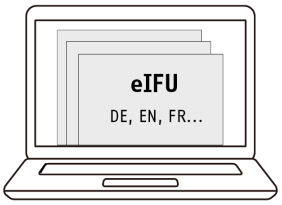
Reagent Kit

B-KSCAL-RSET
Version A1.1

For *In Vitro* Diagnostic Use

Manufacturer

BÜHLMANN Laboratories AG
Baselstrasse 55
4124 Schönenbuch
Switzerland
Tel.: +41 61 487 1212
Fax: +41 61 487 1234
info@buhlmannlabs.ch



www.buhlmannlabs.ch/support/downloads/

INTENDED USE

The BÜHLMANN sCAL® turbo is an *in vitro* diagnostic test designed for the quantitative determination of calprotectin (MRP8/14) in human serum samples. Calprotectin serum levels can be used to determine the inflammatory status of patients. The test is used in combination with clinical chemistry analyzers.

For laboratory use only.

PRINCIPLE OF THE ASSAY

The BÜHLMANN sCAL® turbo test is a particle enhanced turbidimetric immunoassay (PETIA) and allows quantification of calprotectin in human serum samples on clinical chemistry analyzers. Samples can be measured without any additional dilution steps. Samples are incubated with reaction buffer and mixed with polystyrene nanoparticles coated with calprotectin-specific antibodies (immunoparticles). Calprotectin available in the sample mediates immunoparticle agglutination. Sample turbidity, measured by light absorbance, increases with calprotectin-immunoparticle complex formation and is proportional to serum calprotectin concentration. The detected light absorbance allows quantification of calprotectin concentration via interpolation on an established calibration curve.

REAGENTS SUPPLIED

Reagents	Quantity	Code	Preparation
Reaction Buffer (R1) HEPES buffered saline	1 vial 24 mL	B-KSCAL-R1	Ready to use
Immunoparticles (R2) Polystyrene beads coated with polyclonal antibodies against human calprotectin	1 vial 7.3 mL	B-KSCAL-R2	Ready to use

Table 1: Reagents supplied

REAGENT STORAGE AND STABILITY

Unopened reagents
Store at 2-8°C. Do not use kit past expiration date printed on the labels.
On-board stability
Store for up to 55 days at 2-15°C.

Table 2: Storage and stability of reagents

Do not freeze reagents!

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents	Quantity	Code
BÜHLMANN sCAL® turbo Calibrator Kit Calibrators 1-6 for establishment of six-point calibration curve	1 x 6 vials 1 mL/ vial	B-KSCAL-CASET
BÜHLMANN sCAL® turbo Control Kit Controls low and high	3 x 2 vials 1 mL/ vial	B-KSCAL-CONSET

Table 3: Materials required but not provided

WARNINGS AND PRECAUTIONS

- This test is for *in vitro* diagnostic use only.
- It is recommended that the test be handled by qualified personnel, in accordance with Good Laboratory Practice (GLP).
- The immunoparticles contain potentially infectious substances of animal origin and should be handled with caution. Disposal of any discharged materials should be in accordance with local requirements.
- R2 contains polystyrene nanoparticles.
- Unused solution should be disposed according to local state and federal regulations.

Technical precautions

- Do not mix reagents R1 and R2 of different reagent lots or switch caps between reagents.
- Sample carryover depends on the clinical chemistry analyzer. For more information refer to the analyzer specific application note.
- Reagent R2, once frozen, cannot be used anymore. Freezing R2 will lead to reduced sensitivity and precision in low level samples and in the worst case to decreased measurement levels.
- Before measuring please equilibrate reagents, controls, calibrators and samples for 30 min at room temperature before starting analysis.
- Ensure that samples have no bubbles prior to running the test.
- Evaporation of calibrators and controls on the analyzer could lead to incorrect results. Run the assay immediately after loading the analyzer.

SPECIMEN COLLECTION AND STORAGE

Collect blood into tubes without any additives or into gel separator tubes according to manufacturer's instructions and avoid hemolysis.
Centrifuge the venipuncture tube at 1800 x g for 15 minutes at room temperature (18-28°C) and **transfer the serum** into plain tubes **within 6 hours** after blood collection. Avoid co-transferring any blood cells. Do not heat-inactivate samples.

Specimen transport and storage

Separated serum samples can be stored at ambient temperatures up to 28°C for 15 days. Separated samples can be stored at 2-8°C for up to 16 days. For longer storage, keep undiluted serum samples at ≤-20ºC. These samples are stable for at least 1 month at ≤-20ºC. More than 6 freeze-thaw cycles are not recommended.

ASSAY PROCEDURE

Application notes / assay installation

The assay procedure for the BÜHLMANN sCAL® turbo has been established on several clinical chemistry analyzers. Validated application notes describing installation and analysis on specific instruments are available from BÜHLMANN upon request. Corresponding instrument manuals must be considered for instrument setup, maintenance, operation and precautions.

Reagent preparation

The reagents supplied are ready to use. Mix gently before loading onto the instrument. Transfer reagents content to instrument specific bottles.

Establishment of the calibration curve

The BÜHLMANN sCAL® turbo Calibrator Kit is used to establish a six-point calibration curve according to the instrument manual. Calibrator values are lot-specific. A new calibration must be performed for each new calibrator and reagent lot. Otherwise, calibration should be performed every one to two months according to the instrument specific application notes. Refer to the QC-data sheet provided with the BÜHLMANN sCAL® turbo Calibrator Kit for assigned calibrator values. Contact BÜHLMANN support if calibration cannot be performed without error.

QC controls

The BÜHLMANN sCAL® turbo Control Kit must be assayed each day before running patient serum samples to validate the calibration curve. The controls have assigned value ranges indicated on the QC-data sheet supplied with each lot of the BÜHLMANN sCAL® turbo Control Kit. The control measurements must be within the indicated value ranges to obtain valid results for patient serum samples.

If the control values are not valid, repeat measurement with fresh controls. If control values remain invalid, recalibrate the assay. If valid control values cannot be reproduced after performing the steps described above contact BÜHLMANN support.

Patient serum measurement

Once a calibration curve is established and validated with the controls, patient serum may be measured. Perform patient serum measurement according to the application note and instrument manual.

Results

The results are calculated automatically on the clinical chemistry analyzer and presented in µg/mL.

STANDARDIZATION

The BÜHLMANN sCAL® turbo is standardized against internal reference material (purified native calprotectin). Calibrator and control values are assigned according to a value transfer protocol (ref. 1, 2), to guarantee metrological traceability.

The analytical measuring interval of the BÜHLMANN sCAL® turbo, established on the Mindray BS-480 instrument, is 0.23-15.0 µg/mL.

LIMITATIONS

- Lipemic and hemolytic samples show interferences. Please refer to the chapter interfering substances for more information. Lipemic samples can be avoided by asking patients to fast for at least 12 hours prior to drawing the sample.
- Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.

REFERENCE INTERVAL

The reference interval of the BÜHLMANN sCAL® turbo test was established according to CLSI C28-A3 with 160 serum samples from self-declared healthy individuals: adult men and women aged 18 to 83 years. The samples were collected either in native tube or tube with gel separator and processed within 3.4 hours after collection. The results are provided in table 4.

			Reference Interval		
Matrix	**N**	**95% Reference Limit* [µg/mL]**	**2.5 – 97.5 Percentile [ug/mL]**	**90% Confidence Interval**	
				Lower reference limit	**Upper reference limit**
Serum (native tube)	160	1.77	0.24 – 2.01	0.11 – 0.31	1.78 – 2.74
Serum (gel separator)	160	1.10	0.19 – 1.80	0.06 – 0.23	1.10 – 2.07

Table 4: BÜHLMANN sCAL turbo® reference interval; *one-sided

PERFORMANCE CHARACTERISTICS

The presented performance characteristics have been established on a Mindray BS-480 instrument, unless otherwise indicated. Refer to analyzer-specific application notes for the performance characteristics on other clinical chemistry analyzers.

Method comparison:
BÜHLMANN sCAL® turbo vs GCAL® test (Gentian AS)

The method comparison study was performed according to the CLSI guideline EP09-A3. One hundred and eleven (111) samples were measured using 3 lots of BÜHLMANN sCAL® turbo over 3 days. Mean reference values, with calprotectin concentrations of 0.5-10.3 µg/mL falling within the measuring range, were established with 1 lot of the GCAL® test on Roche cobas c501. Bias was determined using Passing-Bablok linear regression and Bland-Altman analysis.

Bland-Altman Analysis			**Passing-Bablok Regression Analysis**		
Mean bias (95% CI)	**Lower LoA (95% CI)**	**Upper LoA (95% CI)**	**Slope (95% CI)**	**Intercept [µg/mL] (95% CI)**	**r**
12.5% (9.8%, 15.1%)	-15.2% (-19.8%, - 10.7%)	40.1% (35.6%, 44.7%)	1.16 (1.13, 1.20)	-0.065 (-0.112, -0.022)	0.990

Reproducibility: 2.9 – 11.1% CV

Reproducibility was established according to the CLSI guideline EP05-A3 using a 3 instrument/lot/operator x 5 days x 5 replicates study design and an acceptance criterion of 15% CV. Testing was performed on Roche cobas c501, Beckman Coulter AU480, and Mindray BS-480 instruments. Four serum samples were assayed.

#	Mean [µg/mL]	n	Within-run		Between-day		Between-lot/ instrument/operator		Total	
			SD [µg/mL]	%CV	SD [µg/mL]	%CV	SD [µg/mL]	%CV	SD [µg/mL]	%CV
1	0.62	75	0.03	5.2	0.01	2.0	0.03	4.6	0.04	7.2
2	1.43	75	0.02	1.5	0.04	2.8	0.09	6.4	0.10	7.2
3	4.03	75	0.06	1.4	0.06	1.5	0.44	10.9	0.45	11.1
4	10.44	75	0.09	0.9	0.12	1.1	0.27	2.5	0.31	2.9

Repeatability: 0.5 – 3.9% CV
Within-laboratory precision: 0.7 – 5.1% CV

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3 using the standardized 20 days x 2 runs x 2 replicates study design. Four serum samples (#1-4) with calprotectin concentrations covering the analytical measuring interval (AMI) (0.66-10.41 µg/mL) of the assay and three serum samples (#5-7) with concentrations covering the extended measuring interval (EMI) (20.62-166.5 µg/mL) were tested.

#	Mean [µg/mL]	n	Repeatability		Between-run		Between-day		Within-laboratory	
			SD [µg/mL]	%CV	SD [µg/mL]	%CV	SD [µg/mL]	%CV	SD [µg/mL]	%CV
1	0.66	80	0.03	3.9	0.02	3.2	0.00	0.0	0.03	5.1
2	1.47	80	0.01	0.9	0.01	0.7	0.00	0.0	0.02	1.2
3	4.16	80	0.04	0.9	0.01	0.3	0.00	0.0	0.04	0.9
4	10.41	80	0.05	0.5	0.05	0.5	0.00	0.0	0.07	0.7
5	20.63	80	0.27	1.3	0.31	1.5	0.19	0.9	0.46	2.2
6	90.88	80	0.75	0.8	0.92	1.0	0.46	0.5	1.27	1.4
7	166.47	80	1.49	0.9	1.92	1.2	0.00	0.0	2.43	1.5

Extended measuring interval (EMI): 15.0 – 225 µg/mL

The extended measuring interval (EMI) of the BÜHLMANN sCAL® turbo was determined according to the CLSI guideline EP34. Samples with known concentration within the EMI were diluted with the recommended dilution factor of 1:15 with demineralized water by one operator on 2 instruments and 2 different reagent lots. Four replicates per dilution on each instrument and for each lot were measured. A maximum dilution recovery of ± 20% was allowed.

Recovery: 97.3 – 104.8%

Six serum samples with calprotectin levels ranging from 0.38 µg/mL to 8.8 µg/mL were spiked with 1.5 µg/mL calprotectin in calibrator material. Spiking was performed at 10% of the specimen volume. “Baseline” samples were spiked with the corresponding volume of analyte-free specimen. “Baseline” and “baseline + spike” samples were measured in four replicates.

Sample carry-over

The sample carry-over was established according to the CLSI guideline EP10-A3. No statistically significant carry-over with the BÜHLMANN sCAL® turbo test on Mindray BS-480 instrument was detected.

Limit of Detection (LoD): 0.11 µg/mL

The LoD was established according to the CLSI guideline EP17-A2 using the classical approach, parametric analysis and a **LoB of 0.07 µg/mL**, determined using a non-parametric analysis.

Lower Limit of Quantitation (LoQ): 0.23 µg/mL

The LoQ was established according to the CLSI guideline EP17-A2, based on 60 determinations and a precision goal of 20% CV.

Linearity range: 0.16 – 15.1 µg/mL

The linear range of the BÜHLMANN sCAL® turbo was determined according to the CLSI guideline EP06-Ed2. For serum calprotectin the method has been demonstrated to be linear from 0.16 to 15.1 µg/mL, within an allowable deviation of ±20% / ±0.2 µg/mL in this interval.

High Dose Hook Effect

Samples with calprotectin concentrations of up to 91.2 µg/mL have been measured on three (3) reagent lots on Mindray BS-480 without limiting the upper limit of quantitation (ULoQ) of the assay.

Interfering substances:

The susceptibility of the BÜHLMANN sCAL® turbo assay to oral and injectable pharmaceuticals, as well as to endogenous substances was assessed according to the CLSI guideline EP07-A3. Bias in results exceeding 20% was considered interference.

Endogenous substances like hemoglobin, intralipid, rheumatoid factors and hemolyzed samples, showed an interference. Samples with rheumatoid factors up to 88.3 U/mL can be measured correct. Above that, wrong positive calprotectin results will occur. Samples with intralipid up to 9.2 mg/mL can be measured. Above that concentration, wrong negative calprotectin results will be measured.

No interference was detected with the following substances up to the listed concentrations: Ibuprofen (0.22 mg/mL), naproxen (0.36 mg/mL), methotrexate (1.36 mg/mL), adalimumab (Humira®) (36 µg/mL), infliximab (Remicade®) (277 µg/mL), tofacitinib (116 ng/mL), prednisone (99 ng/mL), prednisolone (99 ng/mL), dexamethasone (Fortecortin®) (12 µg/mL), ciprofloxacin (12 µg/mL), lansoprazole (1.0 µg/mL), triglyceride (9.2 mg/mL), bilirubin conjugated (0.40 mg/mL), bilirubin unconjugated (0.40 mg/mL) and rheumatoid factors (88.3 U/mL).

REFERENCES

1. Blirup-Jensen et al.: Clin Chem Lab Med 2001; 39, 1110-22
2. Blirup-Jensen et al.: Clin Chem Lab Med 2008; 46, 1470-9
3. Ometto F, et al.: Exp Biol Med 2017; 242(8): 859–873
4. Jarlborg M, et al.: Arthritis Res Ther. 2020; 22(1):105
5. La C, et al. RMD Open 2021;7:e001646

INCIDENT REPORTING IN EU MEMBER STATES

If any serious incident in relation to this device has occurred, please report without delay to the manufacturer and competent authority of your Member State.

SHIPPING DAMAGE

Please notify your distributor, if this product was received damaged.

REACH

None of the materials and reagents in the kit require a Material Safety Data Sheet (MSDS) according to CLP-Regulation (EC) No 1272/2008 and directive EC 1907/2006 (REACH).

SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1.
For definition of symbols see the symbol glossary at:
www.buhlmannlabs.ch/support/downloads/
In addition the following symbols and signs are used:

EN: electronic instruction for use available in different languages at:/ **DE**: elektronische Gebrauchsanweisung in verschiedenen Sprachen verfügbar unter:/ **FR**: un mode d'emploi électronique disponible en différentes langues à l'adresse:/ **IT**: istruzioni elettroniche per l'uso disponibili in diverse lingue su:/ **ES**: instrucciones de uso electrónicas disponibles en diferentes idiomas en:/ **PT**: instrução electrónica para utilização disponível em diferentes línguas em:/ **BG**: електронни инструкции за употреба на различни езици на адрес:/ **DA**: elektronisk brugsanvisning på forskellige sprog på:/ **ET**: elektrooniline kasutusjuhend, mis on saadaval erinevates keeltes aadressil:/ **EL**: ηλεκτρονικές οδηγίες χρήσης διαθέσιμες σε διάφορες γλώσσες στη διεύθυνση:/ **LV**: dažādās valodās pieejama elektroniska lietošanas instrukcija:/ **LT**: elektroninės naudojimo instrukcijos įvairiomis kalbomis:/ **NO**: elektronisk instruksjon for bruk tilgjengelig på forskjellige språk på:/ **PL**: elektroniczna instrukcja obsługi dostępna w różnych językach na stronie:/ **RO**: instrucțiuni electronice de utilizare disponibile în diferite limbi la adresa:/ **SV**: elektronisk bruksanvisning på olika språk på följande adress:/ **SK**: elektronický návod na použitie dostupný v rôznych jazykoch na:/ **CS**: elektronický návod k použití dostupný v různých jazycích na adrese:/ **HU**: különböző nyelveken elérhető elektronikus használati utasítás a következő címen:/ **SR**: elektronsko uputstvo za upotrebu dostupno na različitim jezicima na:

www.buhlmannlabs.ch/support/downloads/

CE