



Flow CAST[®]

Basophil Activation Test (BAT) Flow Cytometry

For *in vitro* diagnostic use.

FK-CCR 100 tests

Release date: 2022-05-23
Version A1

 **Manufacturer**

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INTENDED USE

The BÜHLMANN Flow CAST® is an *in vitro* diagnostic test for the qualitative assessment of basophil activation upon stimulation with specific allergens. The test employs flow cytometry to determine the basophil population expressing CD63 cell surface marker in patient K-EDTA whole blood samples. Flow CAST® is intended as an aid to diagnosis of immediate type allergic disorders in conjunction with other clinical and laboratory findings.

For laboratory use only.

PRINCIPLE OF THE ASSAY

Flow CAST® is a flow cytometry-based basophil activation test (ref. 1, 2). Whole blood from patients is stimulated with specific allergens, as well as stimulation buffer and stimulation controls, to evaluate the patient's basophil degranulation *ex vivo*. The sample is stained using two fluorescently labeled monoclonal antibodies: one for basophil selection (anti-CCR3-PE) and one for basophil activation status determination (anti-CD63-FITC) (ref. 3-6) CD63 is a transmembrane protein present on intracellular vesicles and only presented on the cell surface after basophil degranulation (ref. 7).

Erythrocytes from the patient sample are removed by a lysing reaction. Depending on the protocol, the cells are centrifuged, resuspended in wash buffer and fixed for later analysis by flow-cytometry or analyzed directly after lysis. Basophils are gated from the leukocyte population as CCR3^{pos}/SSC^{low}. The activation status of the gated basophils is determined by their CD63 expression (activation marker). Patients who do not elicit IgE-mediated allergic responses, so called non-responders, are identified based on the results of the positive controls. The readout of the assay is indicated as the ratio of CD63 positive basophils over all basophils (%CD63 activation).

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Comments
Stimulation Buffer containing calcium, heparin and IL-3	1 vial lyophilized	B-CCR-STB	Reconstitute with 50 mL of water ¹⁾
Stimulation Control anti-FcεRI mAb	1 vial lyophilized	B-CCR-STCON	Reconstitute with 1.5 mL of B-CCR-STB
Stimulation Control fMLP ²⁾	1 vial lyophilized	B-CCR-FMLP	Reconstitute with 1.5 mL of B-CCR-STB
Staining Reagent Mix of anti-CD63-FITC and anti-CCR3-PE mAb	1 vial 2.2 mL	B-CCR-SR	Ready to use
Lysing Reagent ³⁾ 10x concentrated	1 vial 25 mL	B-CCR-LYR	Dilute with 225 mL of deionized water
Wash Buffer with 0.1% formaldehyde	1 vial 100 mL	B-CCR-WB	Ready to use

Table 1

¹⁾ For required water quality, refer to chapter Technical Precautions

²⁾ N-formyl-methionyl-leucyl-phenylalanine

³⁾ Crystals may be formed during storage at 2-8°C and should be dissolved at 18-28°C prior to dilution

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 and reconstituted reagents	
Stimulation Buffer	Stable at -20°C for 6 months. Aliquot if repeated use is expected.
Stimulation Control	
Stimulation Control fMLP	
Lysing Reagent	Stable at 2-8°C for 6 months.
Staining Reagent	
Wash Buffer	

Table 2

MATERIALS REQUIRED BUT NOT PROVIDED

- K-EDTA venipuncture tubes
- Centrifuge
- Disposable, pyrogen-free polypropylene or polystyrene flow cytometry test tubes
- Flow cytometry test tube racks for the stimulation
- Vortex mixer
- (Optional) tissue culture grade microtiter plates for cell stimulation and staining for the standard protocol
- (Optional) deep-well plates for cell stimulation, staining, lysis and flow-cytometry acquisition for the lyse-no-wash protocol
- Precision pipettes with disposable, pyrogen-free tips:
 - 10-100 µL, 100-1000 µL,
 - 1-5 mL adjustable pipette and
 - 10-50 µL adjustable dispenser
- 50 mL cylinder for stimulation buffer reconstitution
- Sterile, ultrapure and apyrogenic water for reconstitution of the stimulation buffer (refer to chapter Technical Precautions)
- Water bath (recommended) or incubator set at 37°C
- Distilled or deionized water as well as appropriate laboratory glassware for the dilution of lysing reagent
- Lids or parafilm to cover tubes during incubation steps
- Bottle-top dispensers for lysing reagent and wash buffer
- Flow cytometer equipped with a 488 nm (blue) laser source as well as emission filters for PE and FITC detection
- Flow cytometric analysis software (refer to chapter Flow Cytometric Data Acquisition)

ALLERGENS TO BE ORDERED SEPERATLEY

Allergens validated for Flow CAST® are offered separately by BÜHLMANN. For allergen order codes as well as information on allergen preparation refer to the BÜHLMANN Allergen Booklets on BÜHLMANN's website:

www.buhlmannlabs.ch

Important: The Flow CAST® was tested only in combination with the CAST® Allergens available from BÜHLMANN Laboratories AG. It is the sole responsibility of the laboratory to validate the use of allergens obtained from other sources.

PRECAUTIONS

Safety precautions

- The stimulation buffer (B-CCR-STB) of this test contains components of human origin. Although tested and found negative for HBV surface antigen, HCV and HIV1/2 antibodies, the reagents should be handled as if capable of transmitting infections and should be handled in accordance with Good Laboratory Practices (GLP) using appropriate precautions.
- Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water.
- Reagents and chemicals have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

Technical precautions

- Recommended water quality for the Flow CAST®: The use of sterile, ultrapure and apyrogenic water to reconstitute stimulation buffer (B-CCR-STB) is essential for good and reproducible basophil stimulation. The following sources of water may be used: cell culture grade water, infusion grade water or deionized, double distilled water that is ultra-filtered in a periodically sanitized 10 kDa ultra filter.
- The lysing reagent (B-CCR-LYR) can be reconstituted with deionized, double distilled water or the same water quality that is used for the reconstitution of the stimulation buffer.
- Avoid allergen contamination during cell stimulation: Aeroallergens in the laboratory may contaminate open blood samples and cell suspensions, causing an elevated background. Blood samples and cell stimulation tubes must be carefully covered by lids or parafilm. Avoid house dust mites, pollinating plants, latex gloves or equipment potentially containing latex as well as open windows in the laboratory where the cell stimulation is performed. It is recommended to carry out the cell preparation and stimulation steps in a laminar flow hood.
- A water bath is recommended over an incubator, due to more efficient heat transfer. If using an incubator, verify that the temperature is 37°C. Lower or higher temperatures may affect results.
- Generally low level of basophil activation is expected for drug allergens. It is therefore crucial that optimal conditions during stimulation including temperature are achieved. The use of single tubes instead of deep-well plates is recommended for drug allergens.
- Components must not be used after the expiry date printed on the labels.
- Do not mix different lots of reagents.
- Avoid contamination of reagents.

Test procedure

- Equilibrate the lysing reagent to room temperature (18-28 °C).
- 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 detailed in this instruction for use.
- Samples that are not properly handled may cause inaccurate results.

- Verify the preparations by eye to assess the efficacy of lysis. The erythrocytes may be incompletely lysed and appear on a light diffraction histogram in the same location as the leucocytes.
- Prolonged lysis time can lead to cell loss. Ensure that you have at least 300 basophils for data acquisition. We recommend that acquisition of samples processed with the lyse-no-wash protocol is performed within an hour.
- Flow cytometry may produce false results, if: the cytometer is misaligned, the fluorescence emission has not been appropriately compensated, the gating regions have not been carefully positioned.

SPECIMEN COLLECTION AND STORAGE

It is recommended that patients avoid systemically administered antiallergenic drugs such as corticosteroids, chromoglycic acid (DSCG) for at least 24 hours prior to blood sampling.

Collect blood into **K-EDTA venipuncture tubes**, by filling the tubes up to the dedicated volume mark. Tubes must be filled at least half-way. One (1) mL of whole blood is sufficient for approximately 18 test tubes.

Do not centrifuge or freeze blood samples.

Whole blood

Whole blood samples stored at 2-8 °C should be processed within 48 hours of collection.

For determination of allergic disorders to drugs it is advised to process samples immediately and no later than within 24 hours of sample collection.

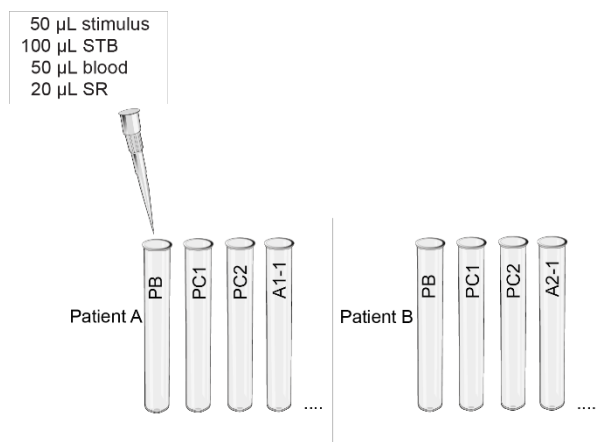
Whole blood samples may also be kept at room temperature (temperatures up to 28 °C). They must however be processed within 24 hours of collection using the standard protocol or on the day of collection using the lyse-no-wash protocol.

Processed samples

Cells processed using the standard protocol are fixed. Fixed cells may be stored at 2-8 °C for 5 days for subsequent acquisition by flow cytometry.

ASSAY PROCEDURE

1. Mix the anti-coagulated blood sample by inverting the venipuncture tube several times.
2. Prepare fresh and pyrogen-free standard polypropylene or polystyrene flow cytometry tubes.
3. For each patient, label the tubes e.g. the following:
PB = patient background
PC1 = stimulation control with anti-FcεRI Ab
PC2 = stimulation control with fMLP
A1-1 for allergen 1 with dilution 1
A1-2 for allergen 1 with dilution 2 etc.



Stimulation and staining

- Add 50 µL of the corresponding stimulus to each tube:
PB tube: 50 µL of **stimulation buffer** (patient background)
PC1 tube: 50 µL of **stimulation control** anti-FcεRI mAb
PC2 tube: 50 µL of **stimulation control** fMLP
Ax-y tube: 50 µL of **allergen**
- Add 100 µL of stimulation buffer (STB) to each tube.
- Add 50 µL of patient's whole blood to each tube. Make sure that the side and top of the tube are free from blood.
- Mix gently.
- Add 20 µL staining reagent (SR) to each tube.
- Mix gently, cover the tubes and incubate for 15 minutes at 37°C in a **water bath**.

Note: If an incubator, instead of a water bath, is used the incubation time is prolonged to 25 minutes due to less efficient heat transfer.

Lysing

Note: The lysing reagent must be equilibrated to room temperature (18-28 °C).

Standard protocol: Lyse and wash

- Add 2 mL equilibrated (18-28°C) lysing reagent to each tube, mix gently.
 - Incubate for 5-10 minutes at 18-28°C.
 - Centrifuge the tubes for 5 minutes at 500 x g.
 - Decant the supernatant by using blotting paper.
 - Resuspend the cell pellet with 300 µL of wash buffer (a fixative is included in the wash buffer).
- Note:** The amount of wash buffer may be adapted to the specific flow cytometer instrumentation used, according to the dead volume and cell density compatible with the device.
- Vortex gently.

Either 16a. acquire the samples on the flow cytometer.

Or 16b. if not acquired immediately, let the samples incubate for 30 min at RT and protected from light (fixation). Store the samples sealed and protected from light at 2-8°C until measurement. Fixed cells may be stored at 2-8 °C for 5 days for subsequent acquisition by flow cytometry.

Note: Stored fixed samples can be acquired without any pre-treatment at any time. Please refer to section "Specimen Collection and Storage" for storage times. A slight decrease of fluorescence intensity and a lower basophil recovery ≥80% may be observed after longer storage.

Alternative protocol: Lyse-no-wash protocol

New generation, high-performance flow cytometers can analyze lysed, unwashed samples. This procedure must be adapted to the flow cytometer instrumentation used and may require optimization. The protocol below is based on data acquired with an attune NxT Flow Cytometer (Thermo Fisher).

- Perform assay procedure steps 1 to 9 (above), and then continue to step 10 below. Add 1.5 ± 0.5 mL equilibrated (18-28°C) lysing reagent to each tube, mix gently (volume must be optimized depending on acquisition speed capabilities of the flow cytometer used).
- Acquire the samples using a high throughput suitable flow cytometer device at high acquisition speed to keep analysis time minimal.

Note: Samples should be analyzed within 24 hours of receiving the sample. Please refer to section "Specimen Collection and Storage".

FLOW CYTOMETRIC DATA ACQUISITION

Flow cytometric acquisition can be performed on any flow cytometer working with a 488 nm argon laser diode (blue-green excitation light).

The flow cytometer must be equipped to detect Forward Scatter (FSC), Side Scatter (SSC) and the two fluorochromes FITC and PE channels.

Ensure that the flow cytometer is properly aligned, and color compensation is set.

For the appropriate acquisition and characterization of resting and activated basophils create the following histograms:

- Create Histogram 1, as Forward Scatter vs Side Scatter to acquire the whole leukocyte population as shown in Figure 1. During acquisition of the samples, make sure that the leukocyte population is separated into three discrete populations (lymphocytes, monocytes and granulocytes) on the FSC/SSC histogram. Adjust the amplification (gain) of FSC and SSC signals to obtain a distribution as shown in Figure 1. Refer to the flow cytometer product manuals for instructions.
- Create Histogram 2, as CCR3-PE vs Side Scatter as shown in Figure 2. Set a gate (e.g., basophils) including the entire basophil population as CCR3^{pos} and SSC^{low} as shown with the rectangle gate in Figure 2. Eosinophils, that are also CCR3^{pos}, must be excluded based on the high SSC.
- Create Histogram 3, as CD63-FITC vs CCR3-PE, showing only the gated basophils, as shown in Figure 3. Use the non-stimulated, resting basophils of the patient background (PB) tube to set a quadrant gate including CD63 negative basophils cells in the lower right quadrant (CD63^{neg} CCR3^{pos}/SSC^{low}) as shown in Figure 3. Basophils activated by the stimulation of positive controls and specific allergens will result in CD63 positive basophil population (CD63^{pos}/CCR3^{pos}/SSC^{low}) identified in the upper right quadrant, as shown in Figure 4 with an example of positive control stimulation (STCON).

The readout of the assay is indicated as the ratio of CD63 positive basophils over all basophils (%CD63 activation) as identified in the quadrant gate of the histogram 3 for any of the stimulation tubes.

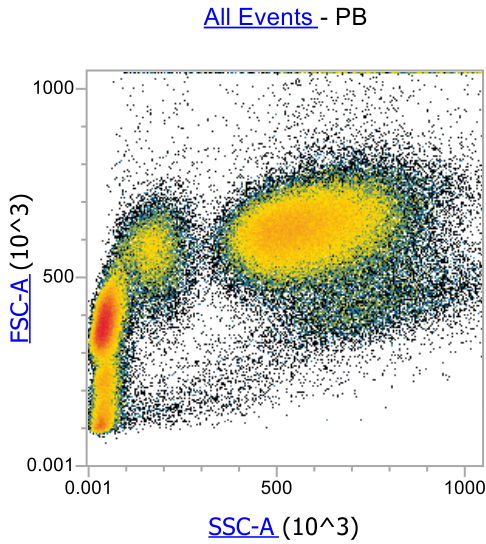


Figure 1: Three discrete populations (lymphocytes, monocytes and granulocytes) on an FSC/SSC histogram.

Acquire 500 or more basophilic cells for any stimulation tubes (gated as shown in Histogram 2, Figure 2 below). If less than 300 basophilic cells are acquired (e.g. in case of basopenia), test results cannot be evaluated.

DATA ANALYSIS

Acquired data is analyzed with appropriate flow cytometry analysis software. Set similar histograms and gates as done for the acquisition.

The gates that identify the basophils in histogram 2 can be independently adapted in any of the different stimulations for the same patient sample.

For the correct evaluation and standardization of the results a background setting for each single patient is defined using the patient background stimulation (PB). The quadrant gate set on the histogram 3 must be set on the PB. To standardize the analysis the gating is set to be between 2 and 2.5% activated basophils (see Figure 3).

This gate must be applied to all subsequent stimulations for the same patients (PC1, PC2, and all measured allergens) to calculate the percentage of CD63 positive cells in any stimulation (see Figure 4).

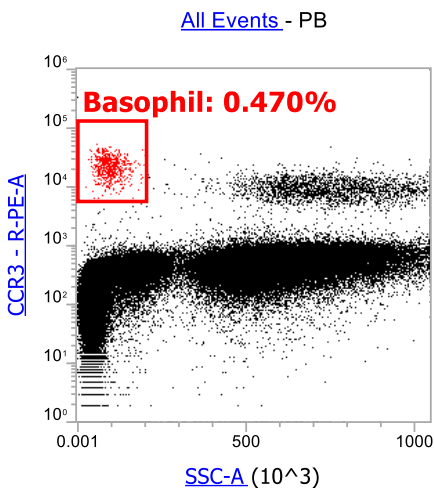
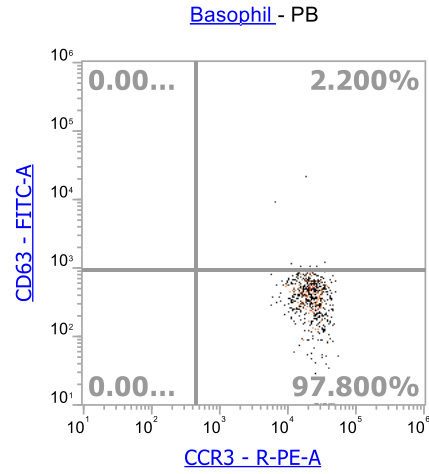
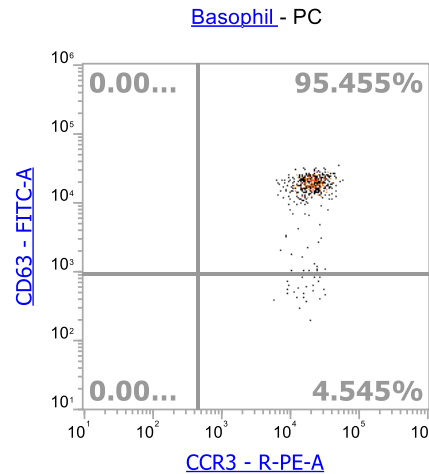


Figure 2: Selection of basophilic cells CCR3^{pos} / SSC^{low}



Gated Region	Count (n=)	%
Total	125'864	100.0
Basophil	591	0.47
Q2 (CD63 ^{pos})	13	2.2
Q4 (CD63 ^{neg})	578	97.8

Figure 3: Patient Background (PB) with STB only



Gated Region	Count (n=)	%
Total	130'926	100.0
Basophil	506	0.386
Q2 (CD63 ^{pos})	483	95.5
Q4 (CD63 ^{neg})	23	4.5

Figure 4: Stimulation Control (STCON)

QUALITY CONTROL

The following criteria and quality control measures should be met for a valid result:

Leukocyte populations: Typically, three distinct leukocyte populations lymphocytes, monocytes and granulocytes should appear in the FSC/SSC plot (see Figure 1). Their occurrence can be regarded as a criterion for the quality of the blood sample (time frame between sample collection and assay execution, storage conditions). Test results cannot be evaluated if less than 300 basophils are acquired.

Stimulation (positive) controls anti-Fc ϵ RI mAb and fMLP: Anti-Fc ϵ RI mAb mimics the bridging of the receptor caused by the allergen *in vivo*. fMLP is a tripeptide causing basophil activation in a non-immunologic way.

- If the Anti-Fc ϵ RI mAb control exhibits a value of $\geq 10\%$

activated basophils, the samples can be evaluated.

- If only the fMLP control shows a signal $\geq 10\%$, but the Anti-Fc ϵ RI mAb does not, the assay has been executed correctly, but test results cannot be evaluated. The patient is considered an IgE non-responder.
- If both Anti-Fc ϵ RI mAb and fMLP exhibit values $<10\%$ activated basophils a technical error is likely. The test result should be considered invalid and the test should be repeated.

STANDARDIZATION

Flow CAST[®] detects the population of basophils expressing the CD63 cells surface marker as % of total basophils. There are no internationally or nationally recognized reference materials or reference measurement procedures for this analyte.

Batch-to-batch reproducibility is guaranteed by titration of anti-CD63-FITC and anti-CCR3-PE monoclonal antibody conjugates against calibration beads. For an estimate of batch-to-batch variation please refer to reproducibility results in section “performance characteristics”.

LIMITATIONS

- Flow CAST[®] test results should be interpreted in conjunction with other clinical and laboratory findings.
- To determine drug-related allergic disorders, BAT testing should be performed within 6 months of the allergic reaction (ref. 8).
- Ensure that at least one to two weeks have elapsed after an allergic reaction before performing BAT testing (ref. 8).
- Negative results obtained for drug allergens should not be used for allergy exclusion.
- It is expected that 5 to 10% of patients will be IgE non-responders. For these patients up-regulation of CD63 expression and a positive result will not be observed. Non-responders can be identified using stimulation controls provided with the Flow CAST[®] test (ref. 9).
- Insufficiently filled K-EDTA tubes (less than half-filled) may lead to false negative results.
- Interference with Flow CAST[®] test results is expected for patients under omalizumab (XOLAIR[®]) therapy (ref. 10).
- Systemically administered antiallergenic drugs such as corticosteroids, chromoglycic acid (DSCG) should be avoided for at least 24 hours prior to blood sampling.

INTERPRETATION OF RESULTS

Flow CAST[®] result categories are the following:

Result	Interpretation
< cut-off	negative
\geq cut-off for one or both dilution of the allergen.	positive

Table 3

CUT-OFF AND REFERENCE INTERVAL

A technical cut-off of 5% activated basophils has been established, where results $\geq 5\%$ CD63^{pos} indicate basophil activation.

For each allergen improved specificity is achieved by using the allergen-specific cut-offs as indicated in the BÜHLMANN Allergen Booklets.

Reference intervals were established according to CLSI C28-A3. One hundred and twenty (120) blood samples from a blood donation center were stimulated with stimulation buffer or Anti-Fc ϵ RI mAb and tested according to standard and lyse-no-wash protocols. Testing was performed over the course of 26 days by three operators with two Flow CAST[®] reagent lots.

Control	Assay Protocol	Reference Interval (90% CI) [% CD63 ^{pos}]	
		2.5th percentile	97.5th percentile
Stimulation Buffer	standard	0.8 (0.5 - 1.2)	4.6 (4.1 - 6.4)
	lyse-no-wash	0.9 (0.6 - 1.0)	4.2 (3.9 - 5.3)
Anti-Fc ϵ RI mAb	standard	18.0 (11.5 - 26.0)	97.7 (96.0 - 98.5)
	lyse-no-wash	13.2 (11.4 - 21.2)	96.4 (94.3 - 97.3)

Table 4

CLINICAL PERFORMANCE

Clinical performance of the Flow CAST[®] was evaluated in a systematic scientific literature review. Eleven peer-reviewed studies identified in a systematic literature search covering a time period up to November 2019, one study on insect venoms not identified in the original search and two studies on peanut allergens published after 2019 were included in the analysis. The studies investigated the ability of Flow CAST[®] to differentiate between subjects with allergic disorders as confirmed by a) patient’s clinical history b) oral food challenge (OFC) or provocation test c) clinical history and laboratory testing (skin prick - SPT, sIgE) or d) clinical history and OTC/ provocation test and non-allergic subjects. Studies using sIgE or SPT as sole clinical reference were excluded. The results are summarized in table 5.

Allergen Group	N studies	Sensitivity median (range)	Allergic subjects (total)	Specificity median (range)	Control subjects (total)
Foods Inhalants	5	92% (81-100%)	311	93% (80-100%)	240
Insect venoms	2	87% (73-89%)	79	96% (95-97%)	39
Drugs	7	55% (0-68%)	227	91% (79-100%)	167

Table 5

PERFORMANCE CHARACTERISTICS

Within-laboratory precision: $\leq 25\%$ CV for stimulus

Repeatability (within-run) and within-laboratory precision were established based on the CLSI guideline EP05-A3 and ISO standard 15197:2013. Four donor blood samples were stimulated with stimulation buffer or stimulation control anti-Fc ϵ RI mAb. For the standard procedure, a 2 operators x 4 days x 1 run x 4 replicates study design was used. For the lyse-no-wash, a 2 operators x 1 day x 4 runs x 4 replicates study design was applied. A replicate corresponds to an independent stimulation reaction and a full assay procedure.

The results for stimulation control anti-FcεRI mAb are summarized in Table 6.

Assay Option	Donor	Mean [%CD63]	n	Within-run [%CV]	Between-day (A) Between-run (B) [%CV]	Total [%CV]
standard	A	34.7	32	8.8%	0.0%	15.9%
	B	90.3	32	1.3%	2.0%	3.6%
	C	82.4	32	1.8%	0.0%	5.1%
	D	91.4	32	1.1%	4.5%	5.0%
lyse-no-wash	E	89.5	32	1.5%	1.1%	1.9%
	F	74.0	32	2.7%	3.5%	6.0%
	G	68.2	32	4.2%	12.5%	15.5%
	H	73.9	32	3.3%	2.9%	5.2%

Table 6

Reproducibility: ≤25% CV for stimulus

Reproducibility was established based on the CLSI guideline EP05-A3 and ISO standard 15197:2013. Four donor blood samples were stimulated with stimulation buffer or stimulation control anti-FcεRI mAb. Samples were assayed at two laboratory sites according to the standard protocol. A 3 instruments/lots x 2 operators x 1 day x 5 replicates study design was applied. A replicate corresponds to an independent stimulation reaction and a full assay procedure. The results for stimulation control anti-FcεRI mAb are summarized in Table 7.

Donor	Mean [%CD63]	n	Within-run [%CV]	Between-operator [%CV]	Between-lot/instrument [%CV]	Total [%CV]
A	91.6	30	1.4%	2.1%	1.9%	3.2%
B	87.6	30	1.7%	1.2%	3.3%	3.9%
C	91.9	30	0.8%	0.9%	2.1%	2.5%
D	96.5	30	0.5%	0.0%	0.8%	0.9%

Table 7

INTERFERING SUBSTANCES

The susceptibility of the Flow CAST® assay to pharmaceuticals, abnormal blood conditions and the K-EDTA sample additive was assessed according to the CLSI guideline EP07-A2. Bias in results exceeding 20% for stimulation Control anti-FcεRI mAb and 20% CD63^{pos} (absolute) for Stimulation Control FMLP was considered interference. No interferences were detected at the stated concentrations with the substances listed in Table 8 at the listed concentrations. Interference was detected with K-EDTA at double K-EDTA venipuncture tube concentration for one donor.

Active component	Test Concentration [µg/mL]
Fexofenadine hydrochloride	1.6
Cetirizine dihydrochloride	4.35
Hydroxyzine dihydrochloride	0.27
Ketotifen	0.6
Montelukast	3.84
Prednisone	1.2
N-Acetyl-L-tryptophan	30
Triglyceride (Intralipid)	20'000
Bilirubin conjugated	400
Bilirubin unconjugated	400
Hemolysis	56'100

Table 8

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INCIDENT REPORTING IN EU MEMBER STATES

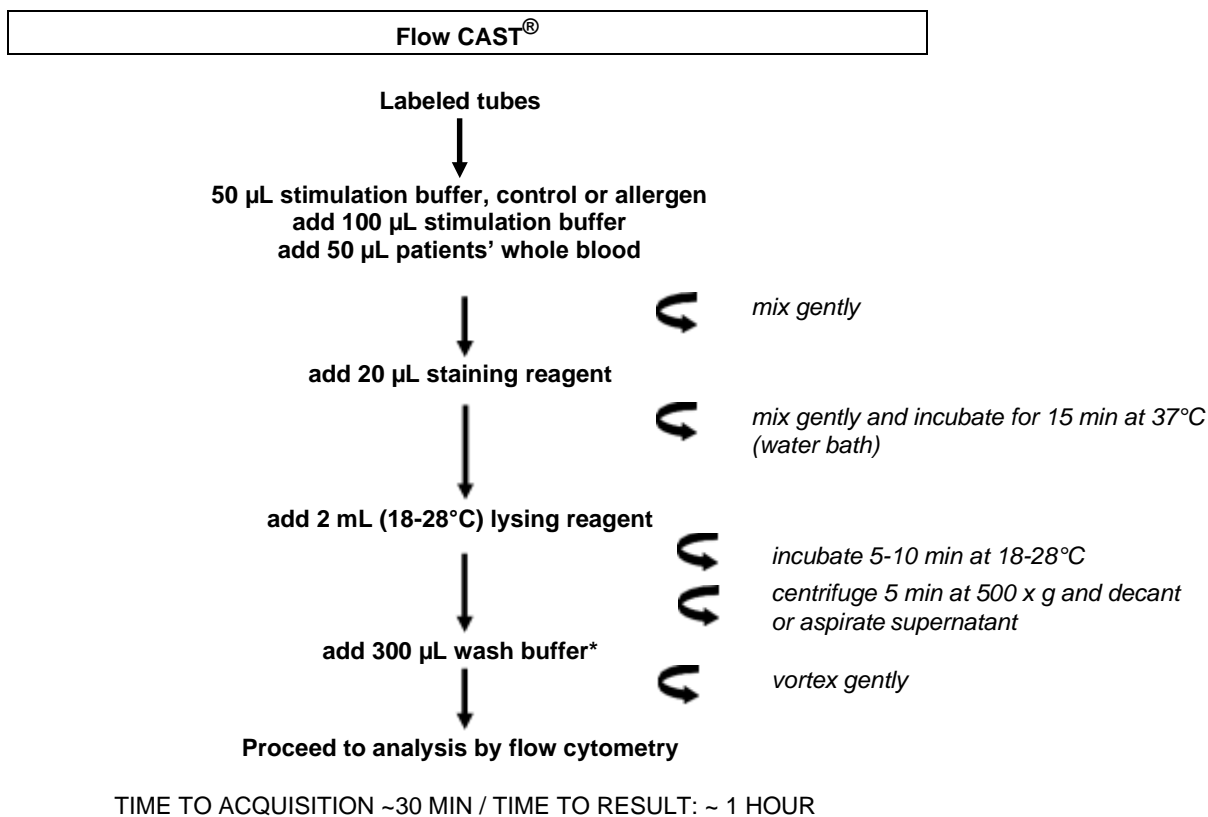
If any serious incident in relation to this device has occurred, please report without delay to the manufacturer and competent authority of your Member State.

SHIPPING DAMAGE

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

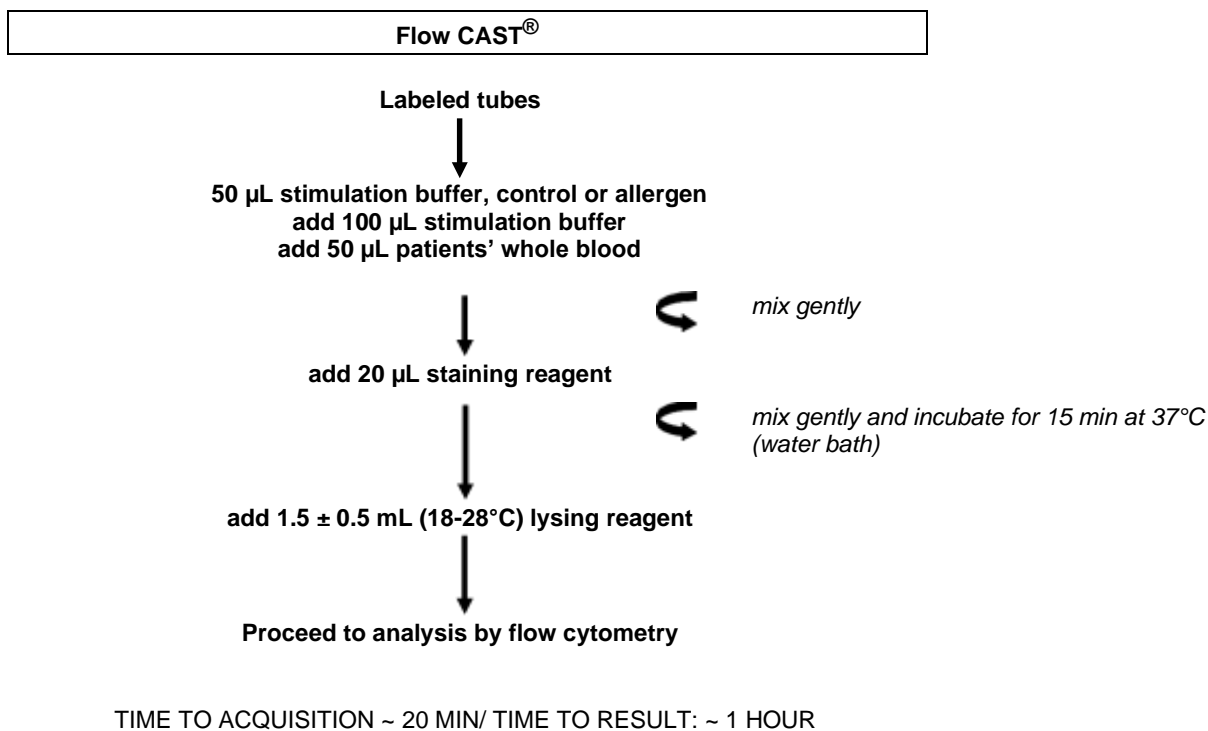
SHORT PROTOCOLS

OPTION A: STANDARD PROCEDURE



* Note: Depending on the flow cytometer instrumentation used, the amount of wash buffer should be adapted in regard on dead volume and cell density compatible with the instrument.

OPTION B: LYSE-NO-WASH PROCEDURE



SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1. In addition the following symbols and signs are used:

Symbol	Explanation
	Stimulation Buffer
	Stimulation Control
	Stimulation Control FMLP
	Staining Reagent
	Lysing Reagent
	Wash Buffer
	<p>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 eletrónica 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:</p> <p style="text-align: center;">www.buhmannlabs.ch/support/downloads/</p>

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