



Flow CAST®

Procedure

FK-CCR

A Commitment to Diagnostics

Pre-Analytics

- Samples required EDTA whole blood
- Sample storage At 2-8°C up to 48 hours (for drug allergy detection only up to 24 hours), do not freeze
- Sample collection Blood has to be drawn before skin testing or *in vivo* provocation. EDTA venipuncture tubes have to be filled >50%
- Patient Patient should discontinue a treatment with drugs such as antihistamines, corticosteroids or chromoglycic acid at least 24 hours before sample collection

Special Equipment

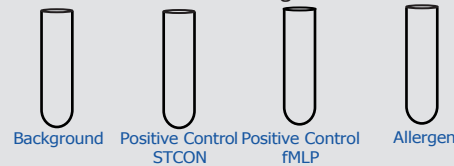
- Water bath, incubator (37°C)
 - Flowcytometer with 488 nm laser diode and the following 4 parameters:
1. Forward scatter, 2. side scatter,
 - 3.-4. Channels for the fluorochromes FITC and PE (FL1, FL2)

BÜHLMANN Allergens

- Resuspend allergens with 250 µl Stimulation Buffer
- Up to 4 stimulations for each allergen vial
- Use freshly reconstituted allergens for stimulation
- Reconstituted Bee & Wasp allergens (BAG2-I1/-I3) are stable for one month stored at -20°C

Stimulation/Staining and Lysis

- Prepare single tubes
- Background
- Positive controls (anti-FcεRI Ab; fMLP)
- Allergens



Add 50 µl Stimulation Buffer (Background) or Positive Control (anti-FcεRI Ab) or Positive Control (fMLP) or Allergen

Add 100 µl Stimulation Buffer

Add 50 µl patients whole blood (EDTA) mix gently

Add 20 µl Staining Reagent mix gently & incubate for 15 minutes at 37°C in a waterbath or 25 minutes in an incubator

Add 2.0 ml pre-warmed (18-28°C) Lysing Reagent incubate 5-10 minutes at 18-28°C

centrifuge 5 minutes at 500 x g and decant or aspirate supernatant

Add 300 µl Wash Buffer* vortex gently

Flow Cytometry Data Acquisition

*Depending on Flow Cytometer in use more wash buffer (e. g. 800 µl) might be needed

Time to result: ~60 minutes

Data Acquisition

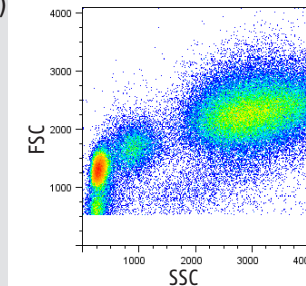


Figure 1: Three discrete populations; lymphocytes, monocytes and granulocytes in FSC/SSC histogram.

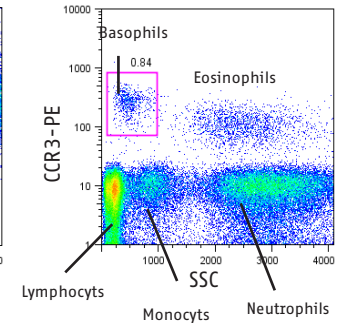
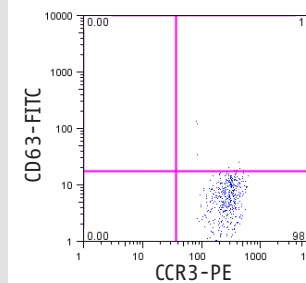
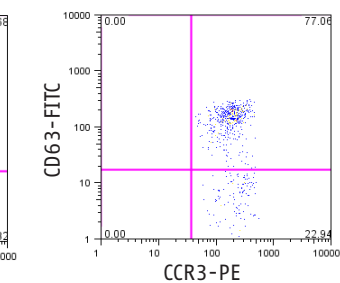


Figure 2: Selection of basophilic cells CCR3^{pos} / SSC^{low}



Gated Region	Count (n=)	%
Total	602	100.0
Q2 (CD63 ^{pos})	11	1.7

Figure 3: Patient Background (PB) with Stimulation Buffer only



Gated Region	Count (n=)	%
Total	650	100.0
Q2 (CD63 ^{pos})	467	77.1

Figure 4: Positive Control (PC) with Stimulation control anti-FcεRI Ab



Flow CAST®

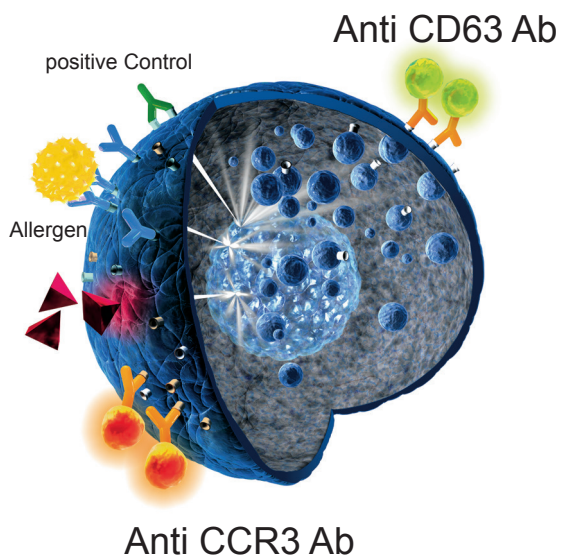
Characteristics

FK-CCR

Intended Use

The Flow CAST® kit is a basophil activation test (BAT) which can be used for the *in vitro* detection of immediate type allergic reactions and hypersensitivities against suspected allergens.

The test is intended for the *in vitro* diagnostic determination of expression of CD63 surface marker on basophils in whole blood by flow cytometry upon allergen stimulation.



Number of samples / per kit

1 Allergen/1 conc.	25 patients
2 Allergen/1 conc.	20 patients
3 Allergen/1 conc.	16 patients

Assay Performance

Specificity

CCR3 is constitutively expressed on eosinophilic and basophilic leukocytes and a smaller part on CD3⁺ cells (lymphocytes). Samples from eight normal blood donors were double stained with anti-CCR3-PE and anti-CD3-AF647. The relative amount (mean) of CD3⁺ cells within the gated Basophil population was 3.9% (95%CI: 2.5-5.2%).

Basophil Recovery

>500 basophils/tube

102 samples from normal blood donors and allergic patients showed a median of 526 cells (95% CI: 481-578 basophils)

Precision (Patient Background)

16.2 %CV

One sample incubated 20 times and subsequently analysed. Mean: 2.4%; SD: 0.4%

Precision (Positive Control)

5.4 %CV

One sample incubated 20 times and subsequently analysed. Mean: 35.5%; SD: 1.9%

Inter Technician Variation

3.7-8.1%CV

Two samples tested by five technicians within the same day:

anti-FcεRI Ab

mean: 69.6%; SD: 2.6%

fMLP

mean: 48.1%; SD: 3.9%

Cut off

Inhalant Allergens ≥15 % CD 63⁺

Food Allergens ≥15 % CD 63⁺

Hymenoptera Venoms ≥10 %CD 63⁺

Betalactams ≥5 %CD 63⁺ SI ≥2

Analgesics ≥5 %CD 63⁺ SI ≥2

Food Additives ≥5 %CD 63⁺ SI ≥2

SI = Stimulation Index: %CD63⁺ Allergen divided by %CD63⁺ Patient Background

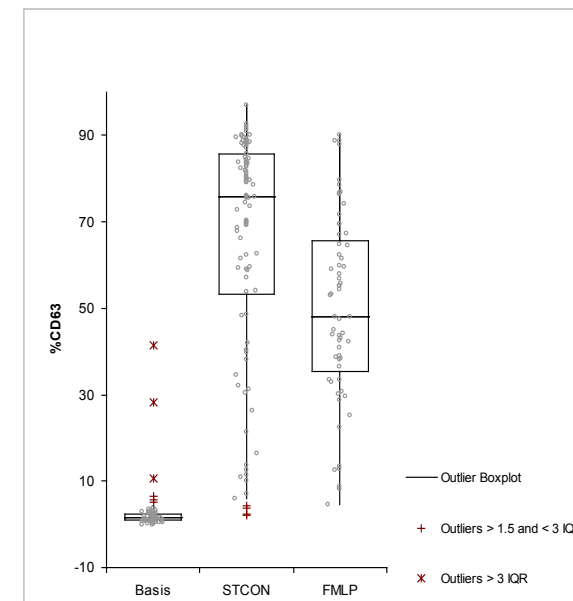


Figure 5: Box Plot Positive and negative controls from normal blood donors. Basis: Negative control (n=98); STCON: positive control anti-FcεRI mAb (n=98); MLP: fMLP positive control (n=61)

CAST® is a registered trademark of BÜHLMANN in many countries.

Ordering code:
FK-CCR 100 tests

CE-marked product



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