

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%

Special Equipment

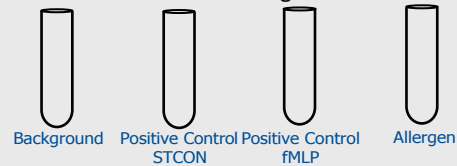
- Water bath, incubator (37°C)
 - Flowcytometer with 488 nm laser diode and the following 4 parameters:
1. Forward scatter, 2. side scatter,
 - 3.-4. Channels for the fluorochromes FITC and PE (FL1, FL2)

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 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

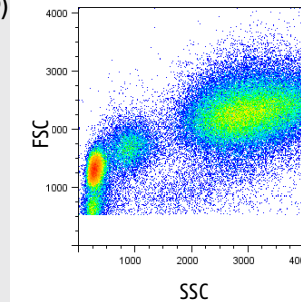


Figure 1: Three discrete populations; lymphocytes, monocytes and granulocytes in FSC/SSC histogram.

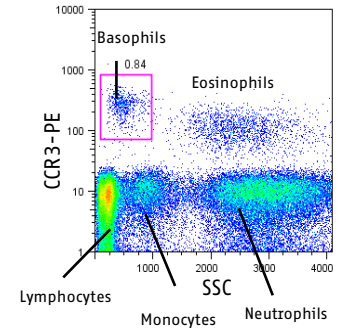
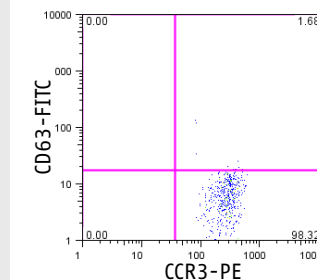
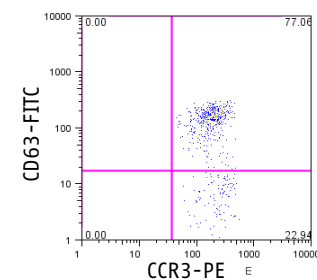


Figure 2: Selection of basophilic cells CCR3^{pos} / SSC^{low}



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

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

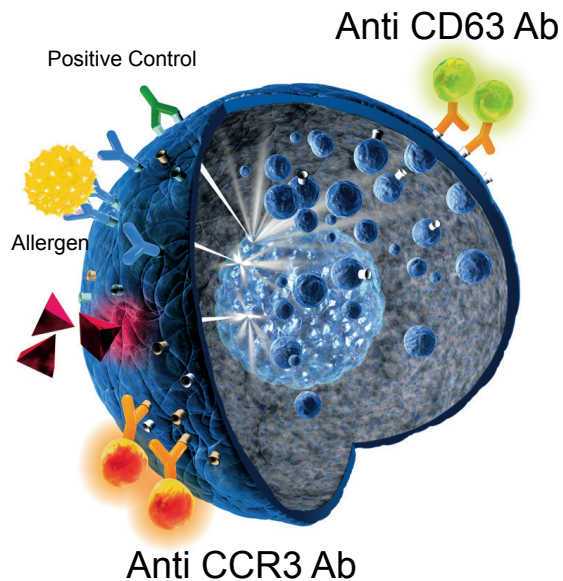


Gated Region	Count (n=)	%
Total	650	100.0
Q2 (CD63 ^{pos})	467	77.1

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

Intended Use

The Flow CAST® kit is a basophil activation test (BAT) intended for the determination of expression of CD63 surface marker on basophils in whole blood by flow cytometry upon allergen stimulation. For Research Use Only. Not for use in diagnostic procedures.



Number of Samples per Kit

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

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 subjects showed a median of 526 cells (95% CI: 481-578 basophils)

Precision (Sample Background)

16.2 %CV

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

Precision (Positive Control)

5.4 %CV

One sample incubated 20 times and subsequently analyzed. 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%

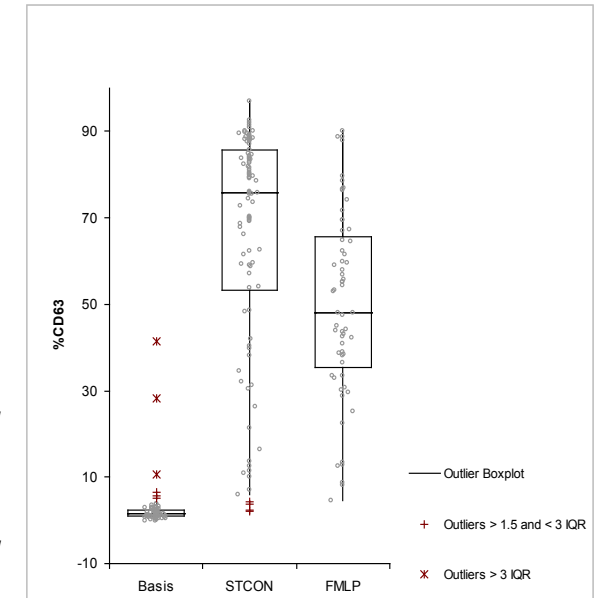


Figure 5: Box Plot positive and negative controls from normal blood donors. Basis: Negative control (n=98); STCON: positive control anti-FcεRI mAb (n=98); MLP: fMLP positive control (n=61)

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

Ordering code:
FK-CCR-U 100 tests

Flow CAST® (FK-CCR-U) is available for Research Use Only in the US and Canada. Not for use in diagnostic procedures.

For Research Use Only in the US and Canada:

Flow CAST® is available for Research Use only in the US and Canada. Not for use in diagnostic procedures.



BÜHLMANN Laboratories AG
Switzerland
info@buhmannlabs.ch
www.buhmannlabs.ch
Phone +41 61 487 12 12

BÜHLMANN Diagnostics Corp
USA / Canada
info@buhmannlabs.com
www.buhmannlabs.com
Phone 844 300 9799