



# 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

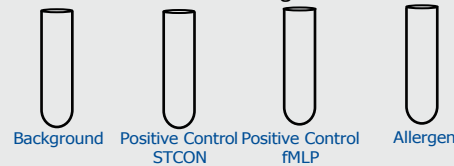
- Water bath, incubator (37°C)
  - Flowcytometer with 488 nm laser diode and the following 4 parameters:
1. Forward scatter, 2. side scatter,
  - 3.-4. Channels for the fluorochromes FITC and PE (FL1, FL2)

### 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

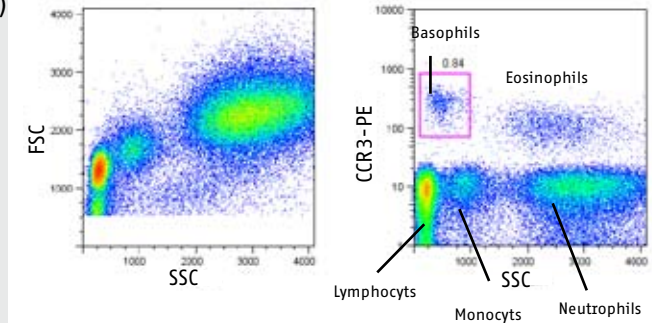
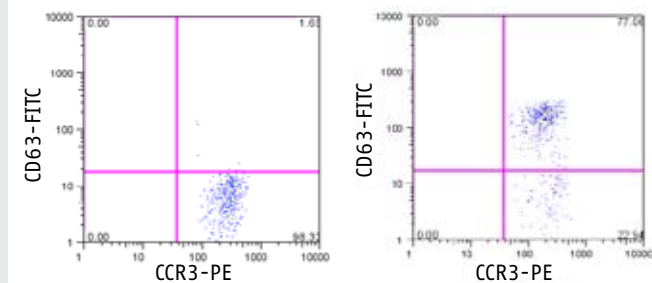


Figure 1: Three discrete populations; lymphocytes, monocytes and granulocytes in FSC/SSC histogram.

Figure 2: Selection of basophilic cells CCR3<sup>pos</sup> / SSC<sup>low</sup>



Gated Region	Count (n=)	%	Gated Region	Count (n=)	%
Total	602	100.0	Total	650	100.0
Q2 (CD63 <sup>pos</sup> )	11	1.7	Q2 (CD63 <sup>pos</sup> )	467	77.1

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

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



# Flow CAST®

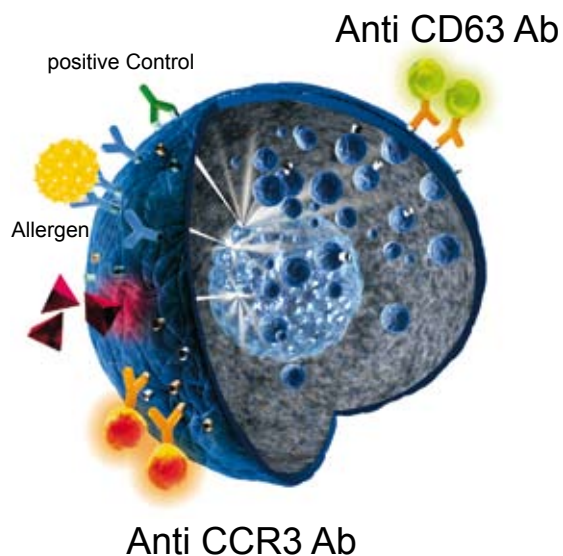
## Characteristics

## 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 antigen 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<sup>+</sup> 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<sup>+</sup> 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 <sup>+</sup>	
Food Allergens	≥15 % CD 63 <sup>+</sup>	
Hymenoptera Venoms	≥10 %CD 63 <sup>+</sup>	
Betalactams	≥5 %CD 63 <sup>+</sup>	SI ≥2
Analgesics	≥5 %CD 63 <sup>+</sup>	SI ≥2
Food Additives	≥5 %CD 63 <sup>+</sup>	SI ≥2

SI = Stimulation Index: %CD63<sup>+</sup> Allergen divided by %CD63<sup>+</sup> Patient Background

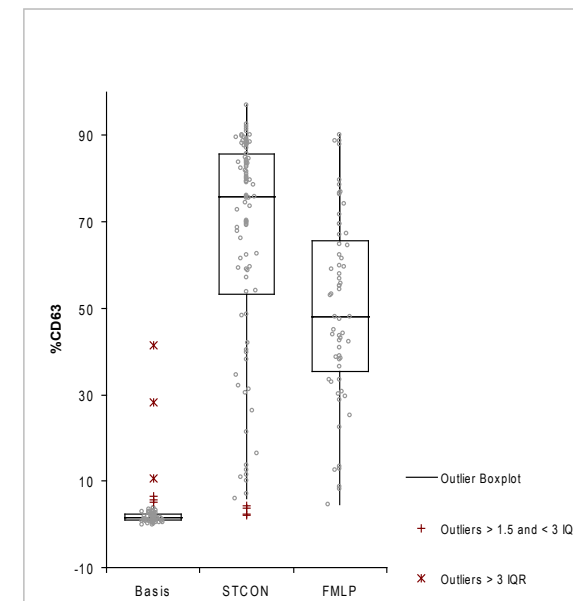


Figure 5: Box Plot Positive and negative controls from normal blood donors. Basis: Negative control (n=98); STCON: positive control anti-FcεRI mAb (n=98); MLP: fMLP positive control (n=61)

Ordering code:  
FK-CCR 100 tests



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