**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

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**FK-CAR Procedure**

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,
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Prepare single tubes

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Add 100 µl Stimulation Buffer

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- 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**

![Data Acquisition Diagram](image-url)
Flow CAST®

**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.

**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

**Number of samples / per kit**

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

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**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); fMLP: fMLP positive control (n=61)

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

**Ordering code:**

FK-CCR 100 tests

- marked product

**Supplied by:**

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