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-FcRI Ab; fMLP)
  - Allergens

**Add 50 µl Stimulation Buffer (Background) or Positive Control (anti-FcRI 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**
**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.

**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)**

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

**Precision (Positive Control)**

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%

**Number of Samples per Kit**

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

**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)

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**Ordering code:**

FK-CCR-U 100 tests

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