



# Flow CAST®

Diagnosis of immediate  
type allergic reactions  
by flow cytometry

User friendly  
1 hour test procedure  
Dual staining protocol

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# Flow CAST®

## Cellular Allergen Stimulation Test by flow cytometry

**BÜHLMANN Flow CAST® is the only CE-marked Basophil Activation Test (BAT) available in combination with >150 standardized and validated allergens.**

### Why Flow CAST®?

According to the World Allergy Organization, worldwide about 25% of the population suffer from allergies which can severely affect the patients' daily routine. Reliable identification of the allergen has to be achieved before effective therapy can be initiated. Since decades the most commonly used diagnostic tools for immediate type allergies have been skin test and specific IgE. However, allergologists are often confronted with unclear and inconclusive results, e.g. due to cross-reactivities, low sensitivity or non-IgE mediated allergies. In such cases, up to now a time-consuming, expensive and potentially high risk procedure, the provocation test, had to be carried out.

BÜHLMANN now offers an easy, fast (1h), reliable whole blood based *in vitro* diagnostic solution for allergy detection by flow cytometry.

Some immediate-type allergies lead to life-threatening anaphylactic reactions (8-10 cases per 100000). The most often presumed etiology of these anaphylactic reactions is caused by food, insect venoms and drugs. In these specific fields of allergy Flow CAST® is at the forefront of diagnosis. Recent publications confirm the benefit of Flow CAST® with excellent sensitivity and specificity [1-8].

### How does the Flow CAST® work?

Upon allergen contact, sensitised basophils degranulate and express the cell surface marker CD63. By labelling specific antibodies against these surface markers to fluorochromes their relative number can be measured with any four parameter flow cytometer (side scatter, forward scatter, fluorescence 1 and 2).

1. Selection of basophils: Basophils constitutively express the eotaxin receptor CCR3 allowing for a robust and accurate selection of cells [11]. CCR3 is measured via a PE-fluorescence labelled antibody (anti-CCR3-PE).

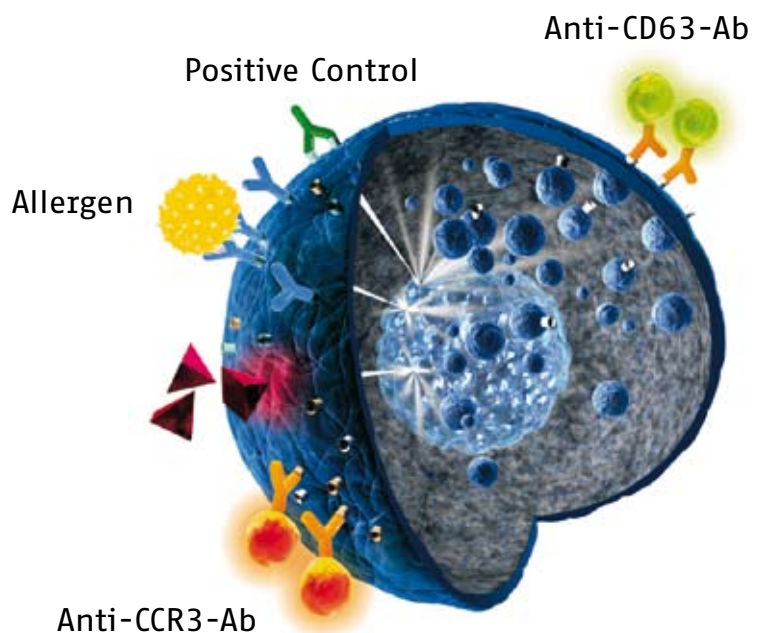
2. Activation of basophils: CD63 is currently the best-validated basophil activation marker directly linked to basophil degranulation. It is detected by a FITC-fluorescence labelled antibody (anti-CD63-FITC).

Two Stimulation controls: The first control, anti-FcεRI, is a highly specific monoclonal antibody binding to IgE binding receptor. The second control, a tripetide (fMLP) activates the basophils by a non-immunologic way.

### What is Flow CAST® highsens?

This novel assay includes in addition to CD63 also CD203c as activation marker (anti-CD63/anti-CD203c-PE-DY647). The CD63 and CD203c markers on the basophils are being upregulated upon allergen contact.

The use of an additional marker CD203c results in a higher sensitivity, especially in drug allergy diagnosis.



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## Clinical case reports

### Amoxicillin Allergy:

Patient: 46 years, male

Anamnesis: Developed severe urticaria in reaction to Amoxicillin in February 2007

Skin Test: Negative

sIgE: Negative

Flow CAST®: Positive for PPL, MDM, Amoxicillin

(Data courtesy of Prof.M.Blanca, Dr.L.Mayorga, Carlos Haya Hospital, Malaga, ES)

The only *in vitro* test detecting the true culprit allergens for severe urticaria was Flow CAST®.

### Cefuroxim Allergy:

Patient: 61 years, female

Anamnesis: Developed an anaphylactic shock in reaction to Cefuroxim

Skin Test: Positive for Cefuroxim, Ciprofloxacin

sIgE: Negative

Flow CAST®: Positive for Cefuroxim, Ciprofloxacin

(Data courtesy of Prof.B.Eberlein, TU München, DE)

Although an anaphylactic shock emerged, sIgE could not be detected for any of the tested antibiotics. Only skin test and Flow CAST® showed positive results and confirmed the anamnesis.

### Peanut Allergy:

Patient: 15 years, male

Anamnesis: Suspected allergy to peanut

sIgE positive: Peanut, Sesam, Soy, Wheat

Flow CAST®: Positive for Peanut

Provocation Test: Positive for Peanut

(Data courtesy of Dr.S.Seneviratne, A.Snorton, St.Mary's Hospital, London, UK)

Low specificity of the sIgE determination led to multiple false positive results. A very strict diet which severely affected the life quality of the patient was administered. With Flow CAST® the only true culprit allergen was found. The result was confirmed by the provocation test.

### Bee & Wasp Allergy:

Anamnesis: Patient's culprit allergen was not identified. Season: summer.

Skin Test: Positive: Wasp

sIgE positive: Bee and Wasp

CCD: Negative

Flow CAST®: Positive: Wasp

(Data courtesy of Dr.O.Hausmann, Clinical Immunology and Allergology, Inselspital Bern, CH)

Double positivity in sIgE measurement is caused by low specificity and a method specific pitfall. BAT with bee and wasp Venom Check Allergens from BÜHLMANN discriminate efficiently in cases of double positivity [7].

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## Publications about Flow CAST®:

### FOOD ALLERGY:

"...a valuable tool in the management of paediatric cow's milk allergy...by contributing in determining whether an oral challenge can safely be undertaken." (Sensitivity and specificity: Flow CAST® 91% and 90% resp., sIgE 94% and 21% resp.) [3]

### INSECT VENOM ALLERGY:

"... a useful in vitro tool in identifying the culprit insect venom in case of double positivity to both wasp and honey bee venom." [7]

"A decreased basophil responsiveness could be demonstrated in bee venom allergic patients after Venom Immunotherapy (VIT) compared to patients before VIT." [8]

### DRUG ALLERGY:

"sensitivity 55%...specificity 80%...the value of two different BAT\* as cellular tests in the in vitro diagnosis of patients with antibiotic allergy...". [1]

\* first and second generation of Flow CAST®

For more information about different allergies, please have a look at the BÜHLMANN insect venom or drug allergy flyer.

## Flow CAST®

**Easy**      two colour assay  
**Fast**        whole blood test  
**Reliable**   cell selection via CCR3

### Literature:

1. B.Eberlein et al.: A new basophil activation test using CD63 and CCR3 in allergy to antibiotics. Clin & Exp Allergy 2010, 40: 411-8.
2. K.Scherer et al.: Cellular in vitro assay in the diagnosis of hymenoptera venom allergy. Intl Arch Allergy and Immuno 2008, 146: 122-32.
3. A.Rubio et al.: Benefit of basophil activation test in deciding when to reintroduce cow's milk in allergic children. Allergy 2010, 66: 92-100.
4. A.Carroccio et al.: A cytologic assay for diagnosis of food hypersensitivity in patients with irritable bowel syndrome. Clin Gastroenterol Hepatol 2010, 8: 254-60.
5. M.L.Sanz et al.: A new combined test with flowcytometric basophil activation and determination of sulfidoleukotrienes is useful for in vitro diagnosis of Hypersensitivity to Aspirin and other nonsteroidal anti-inflammatory drugs. Int Arch Allergy Immunol 2005, 136: 58-72.
6. M.J.Torres et al.: The diagnostic interpretation of basophil activation test in immediate allergic reactions to betalactams. Clin Exp Allergy 2004, 34: 1768-75.
7. O.Hausmann et al.: Double positivity in insect venom allergy – diagnostic approach with basophil activation test. Poster, EAACI 2009.
8. O.Hausmann et al.: Usefulness of the basophil activation tests in monitoring the immune response to bee venom immunotherapy controlled by sting challenge – pilot phase results. Poster, EAACI 2008.



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Ordering code:  
Flow CAST®  
CAST® ELISA

FK-CCR 100 tests  
EK-CAST 192 wells  
EK-CAST5 480 wells

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