

# Flow CAST®

## Intended Use

The Flow CAST® is an in vitro **diagnostic** test for the **qualitative** assessment of basophil activation upon stimulation with specific allergens. The test is intended for the aid of diagnosis of immediate type in conjunction with other laboratory tests.

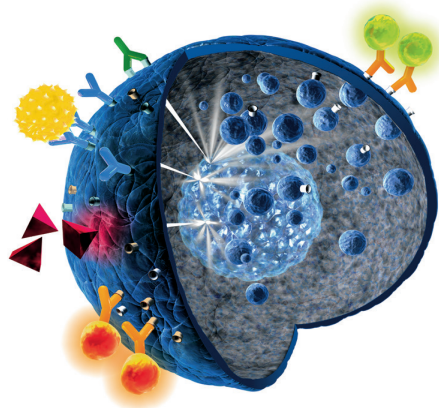
For laboratory use only.

## Principle of the Assay

Flow CAST® is a **basophil activation test (BAT)** that reproduces an allergic reaction in a blood test. At the core of the assay is the ex vivo stimulation of basophils in a patient's whole blood with specific allergen, followed by flow cytometric determination of basophil activation.

Basophils are stained using two fluorescent labelled monoclonal antibodies: the **anti-CCR3-PE** for selection and the **anti-CD63-FITC** for the determination of the activation status. Basophils are identified by gating as CCR3pos/SSC<sub>low</sub>, and their activation status is assessed through CD63 expression.

The **assay's readout** is indicated as the ratio of CD63 positive basophils to all basophils (%CD63).



## Interpretation of Results

Result	Interpretation
< cut-off	negative
≥ cut-off for one or both dilution of the allergen	positive

## Cut-off

- **Technical cut-off is 5%** as established by reference range study
- Allergen-specific cut-off with improved specificity (CAST® Allergen Booklet)

## Clinical Performance

Allergen Group	Number of studies	Sensitivity median (range in %)	Allergic subjects (total)	Specificity median (range in %)	Control subjects (total)
Food Inhalants	5	92 (81-100)	311	93 (80-100)	240
Insect venoms	2	87 (73-89)	79	96 (95-97)	39
Drugs	7	55 (0-68)	227	91 (79-100)	167

11 peer-reviewed studies confirmed effectiveness in distinguishing between individuals with allergic disorders and non-allergic subjects. Allergic conditions were verified through patient's clinical history, oral food challenges, laboratory tests (such as skin prick tests and sIgE), or a combination of these methods.

## Performance Characteristics

### Basophil Recovery

> 500 basophils/tube

### Within-laboratory precision

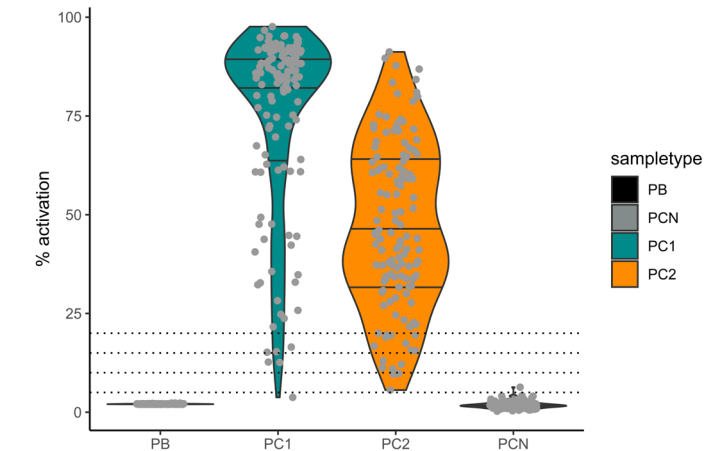
< 25 % CV for stimulus

Performed according to CLSI guideline EP05-A3 and ISO standard 15197:2013

### Reproducibility

< 25% CV for stimulus

Performed according to CLSI guideline EP05-A3 and ISO standard 15197:2013.



## Rate of IgE non-responder below 5%

In a study of 130 healthy blood donors, only 1 person had a poor response to FcεRI receptor crosslinking, resulting in a non-responder rate of 0.77%. Most donors (81.5%) showed a strong response with an activation rate exceeding 50%.

## Numbers of samples / per kit

Number of allergens	Patients
1 / 1 conc.	25
2 / 1 conc.	20
3 / 1 conc.	16

## Pre-Analytics

- Sample required** • EDTA whole blood
- Sample storage** • Store at 2 – 8°C (do not freeze)  
• Process within 48h  
• Drug allergens: Process within 24h
- Collection** • K-EDTA venipuncture tubes must be filled at least half-way

No interference for the most used antihistamine were detected up to a specified concentration, for detail refer to the IFU.

## Processed Specimen Storage

Processed cells using standard protocol are fixed, Fixed cells may be stored at 2-8°C for 5 days for subsequent acquisition.

## Equipment

- Flow cytometer equipped with a 488 nm (blue) laser source with emission filters for PE and FITC channel
- Water bath or incubator (37°C)
- Centrifuge

## CAST® Allergens

\*ordered separately, refer to allergen booklet

### Preparation:

- Resuspend allergens with 250 µl Stimulation Buffer
- Up to 4 stimulations for each allergen vial
- Use freshly reconstituted allergens for stimulation

## Sample Preparation Layout

For each patient prepare single tubes for controls and desired allergens under examination.

PB = Patient background

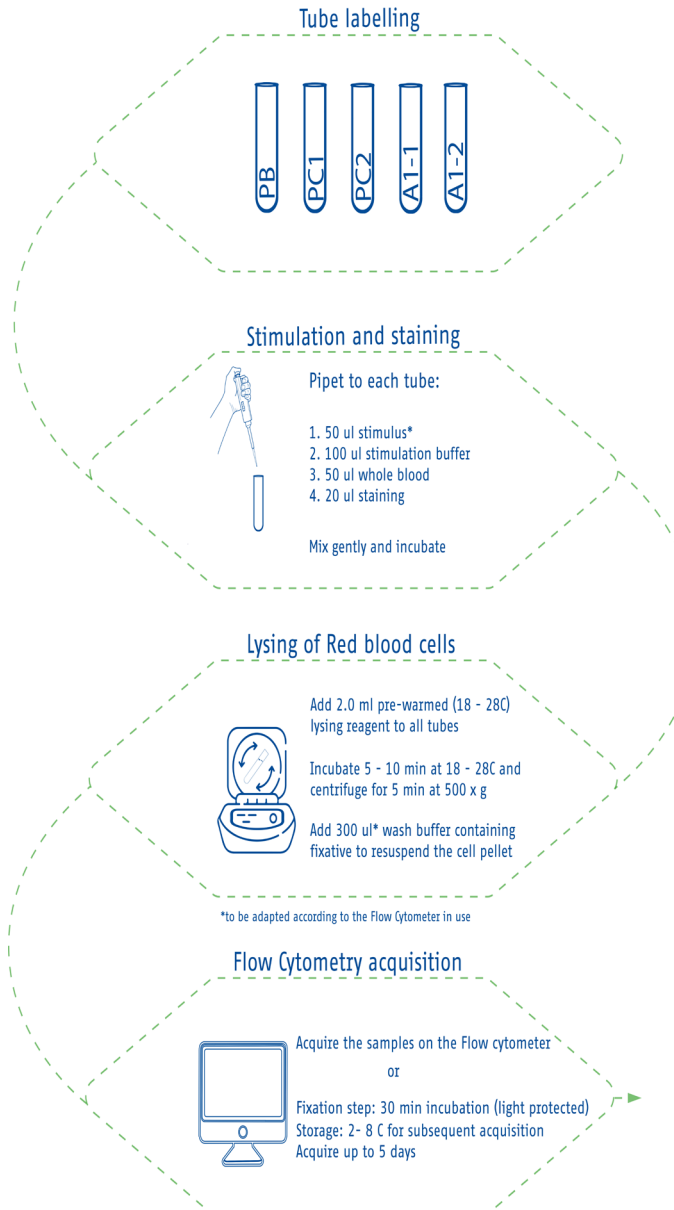
PC1 = Stimulation control with anti-Fcε (epsilon sugn) RI Ab

PC2 = Stimulation control with fMLOP

A1-1 = Allergen 1 with dilution 1

A1-2 = Allergen 1 with dilution 2 etc.

## Procedure (~ 60 minutes)



## Data Acquisition and Interpretation

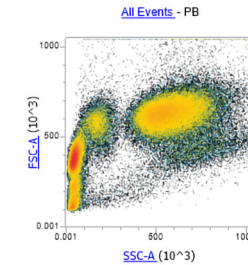


Figure 1: Three discrete populations (lymphocytes, monocytes and granulocytes) on an FSC/SSC histogram

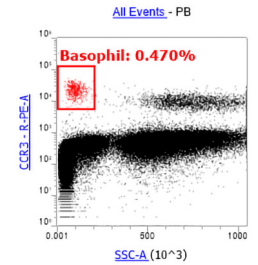


Figure 2: Selection of basophilic cells CCR3<sup>pos</sup>/SSC<sup>low</sup>

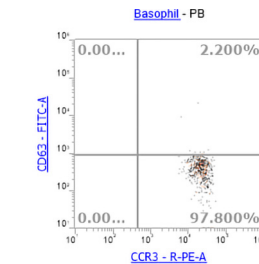


Figure 3: Sample Background (PB) with STB only

Gated region	Count (n=)	%
Total	125'864	100.0
Basophil	591	0.47
Q2 (CD63 <sup>pos</sup> )	13	2.2
Q4 (CD63 <sup>neg</sup> )	578	97.8

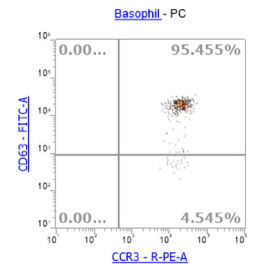


Figure 4: Stimulation Control (STCON)

Gated region	Count (n=)	%
Total	130'926	100.0
Basophil	506	0.386
Q2 (CD63 <sup>pos</sup> )	483	95.5
Q4 (CD63 <sup>neg</sup> )	23	4.5

This document is for information purpose only, before performing the assay please carefully refer/read the respective IFU available (<https://www.buhlmannlabs.ch/support/downloads/eifus/>).

**Ordering code:**  
FK-CCR 100 tests

CE 0123

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