

Flow CAST®

Intended Use

The Flow CAST® is a Research Use Only test for the assessment of basophil activation upon stimulation with specific allergens. The test employs flow cytometry to determine the percent of basophil activation by measuring the number of basophils expressing the CD63 cell surface marker in K-EDTA whole blood samples.

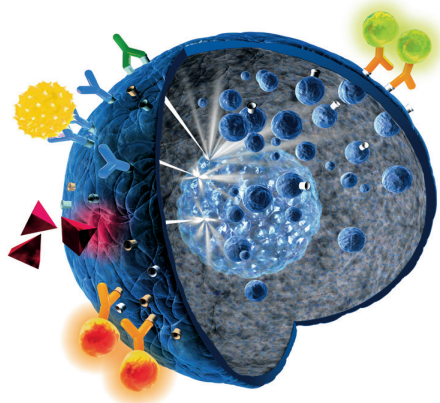
For laboratory use only.

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 an individual's whole blood with specific allergen, followed by flow cytometric determination of basophil activation.

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

The assay's readout is indicated as the ratio of CD63 positive basophils to total 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 (Per CAST® Allergen List)

Clinical Study 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 individuals. Allergic conditions were verified through individual's clinical history, oral food challenges, laboratory tests (such as skin prick tests and sIgE), or a combination of these methods.

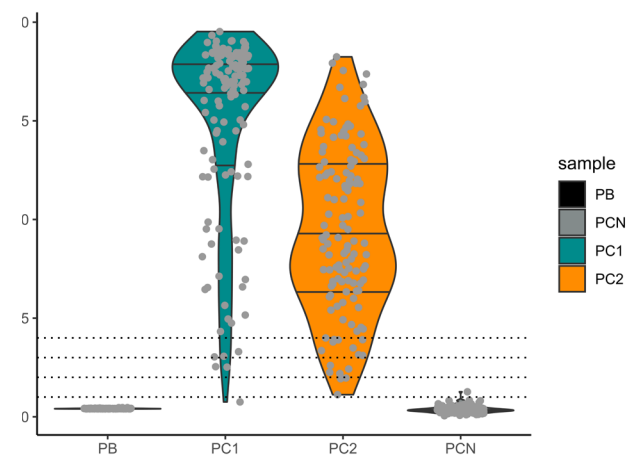
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Performance Characteristics

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 FcεR1 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	Individuals
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 from the most common antihistamine were detected up to a specified concentration. For further 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
- Polystyrene or polypropylene tubes
- Sterile or ultrapure water

CAST® Allergens

*ordered separately, refer to allergen list

Preparation:

- Add 250 µl Stimulation Buffer to prepared allergens
- Use freshly reconstituted allergens for stimulation
- Use up 4 stimulations for each allergen vial

Sample Preparation Layout

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

PB = Sample Background

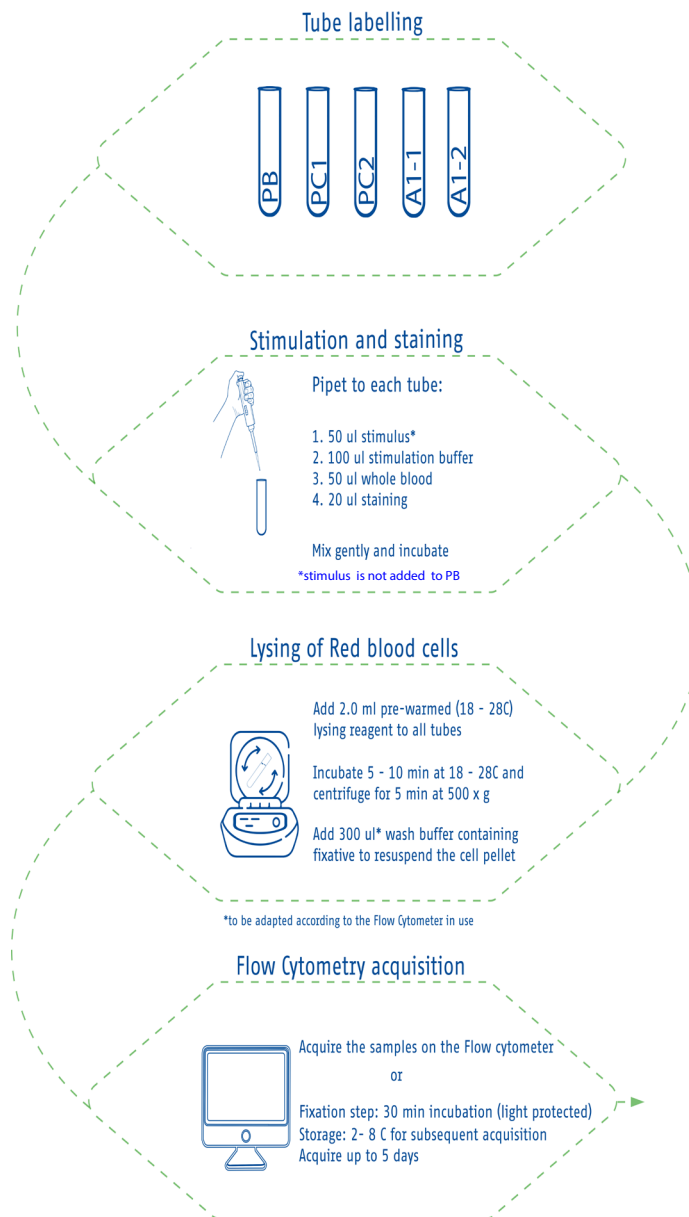
PC1 = Stimulation control with anti-FcεRI AbFc

PC2 = Stimulation control with fMLP

A1-1 = Allergen 1 with dilution 1

A1-2 = Allergen 1 with dilution 2 etc.

Procedure (~ 60 minutes)



Data Acquisition and Interpretation

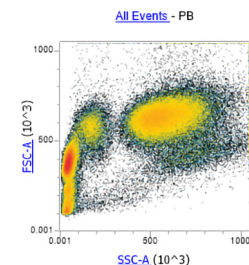


Figure 1: Three discrete populations (lymphocytes, monocytes and granulocytes) on an FSC/SSC histogram

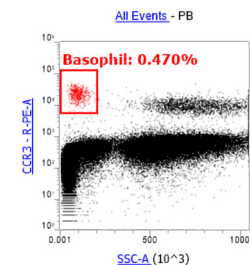


Figure 2: Selection of basophil cells CCR3^{pos}/SSC^{low}

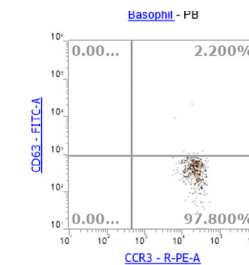


Figure 3: Sample Background (PB) with STB only

Gated region	Count (n=)	%
Total	125'864	100.0
Basophil	591	0.47
Q2 (CD63 ^{pos})	13	2.2
Q4 (CD63 ^{neg})	578	97.8

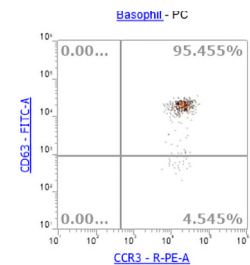


Figure 4: Stimulation Control (STCON)

Gated region	Count (n=)	%
Total	130'926	100.0
Basophil	506	0.386
Q2 (CD63 ^{pos})	483	95.5
Q4 (CD63 ^{neg})	23	4.5

This document is for information purpose only, before performing the assay please carefully refer/read the respective IFU available (<https://www.buhmannlabs.com/cellular-allergy/flow-cast>)

Ordering code:

FK-CCR-U 100 tests



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