



Flow CAST[®] highsens

Procedure

FK-HSBAT

A Commitment to Diagnostics

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 >75% as higher EDTA concentrations inhibit CD63 & CD203c expression
- Patient Patient should discontinue a treatment with drugs such as corticosteroids or chromoglycic acid at least 24 hours before sample collection

Special Equipment

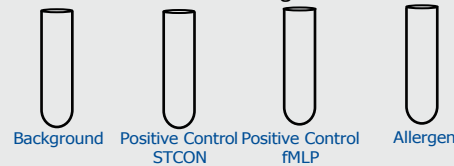
- Water bath, incubator (37°C)
- Flowcytometer with 488 or 532 nm laser diode and the following 4 parameters:
 1. Forward scatter, 2. side scatter,
 - 3.-4. Channels to measure the fluorochromes PE and PE-DY647 (FL2, FL3)

BÜHLMANN Allergens

- Resuspend allergens with 250 µl Stimulation Buffer
- Up to 2 stimulations for each allergen vial
- Use freshly reconstituted allergens for stimulation
- For more information refer to www.buhlmannlabs.ch

Stimulation/Staining and Lysis

- Prepare single tubes
 - Background
 - Positive controls (anti-FcεRI Ab; fMLP)
 - Allergens



Add 100 µl Stimulation Buffer (Background) or Positive Control (anti-FcεRI Ab) or Positive Control (fMLP) or Allergen

Add 200 µl Stimulation Buffer

Add 100 µl patients whole blood (EDTA) mix gently

Add 40 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 Lysing Reagent adapted to 18-28°C incubate 5-10 minutes at 18-28°C

centrifuge 5 minutes at 500 x g and decant or aspirate supernatant

Add 200 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 & Analysis

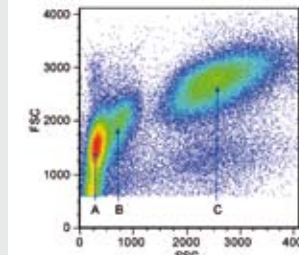


Figure 1: Three discrete populations for lymphocytes (A), monocytes (B) and granulocytes (C) in FSC/SSC histogram.

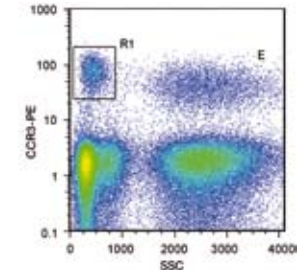
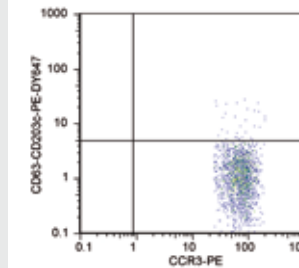
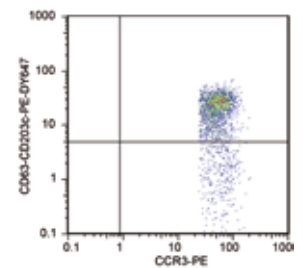


Figure 2: Selection of basophilic cells (R1) CCR3^{pos} / SSC^{low}, Eosinophils (E)



Gated Region	Count (n=)	%
Total	152483	
R1	1560	100.0
Q2 (CD63+ CD203c ^{pos})	33	2.1

Figure 3: Patient Background (PB) with Stimulation Buffer only



Gated Region	Count (n=)	%
Total	156543	
Q2 (CD63 ^{pos})	1504	100.0
Q2 (CD63+ CD203c ^{pos})	1221	81.2

Figure 4: Positive Control (PC) with Stimulation control anti-FcεRI Ab



Flow CAST[®] highsens

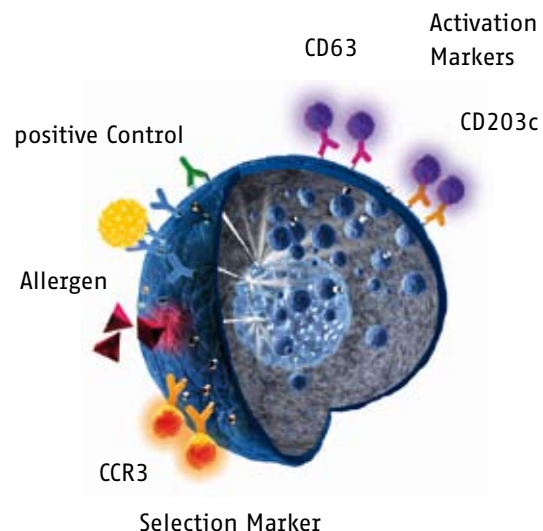
Characteristics

FK-HSBAT

Intended Use

The Flow CAST[®] highsens kit is a basophil activation test (BAT) intended to be used for the in vitro detection of immediate type allergic reactions and hypersensitivities against suspected allergens.

It has been designed to determine the relative amount of CD63 and CD203c positive basophils from their total number (CCR3 positive) in whole blood by flow cytometry upon antigen stimulation.



Assay Performance

Reference Intervals

Reference intervals were established with normal blood donors. Median values for anti-FcεRI and for fMLP stimulated samples are listed below:

	n	% CD63+CD203c pos	
		Median	2.5 th -97.5 th percentile
STCON	116	71.1	3.4 - 97.9
fMLP	70	45.7	8.9 - 89.4

Precision

20 samples were incubated 12 times with stimulation buffer or stimulation control and analyzed by flow cytometry. The precision profile is shown below. At basophil activation above 10% the CV is below 15%.

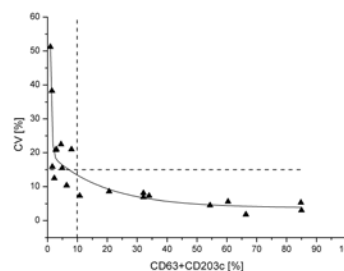


Figure 5: Precision profile: Expressed as coefficient of variation (%CV), plotted against percent activation (CD63⁺CD203c^{pos}).

Inter Technician Variation

6.9-11.7 %CV

Two asymptomatic blood donors were tested by six technicians within the same day:

anti-FcεRI Ab
fMLP

6.9 - 7.0 % CV
6.1 - 11.7% CV

Interferences

Interferences of common anti-histamins have not been detected up to the following concentrations:

Drug	Active component	No interference up to	
Telfast [®]	Fexofenadine	1.9	µg/mL
Aerius [®]	Des-Loratadine	4.0	µg/mL
Claritin [®]	Loratadine	0.017	µg/mL
Cetirizin-Teva [®]	Cetirizine	1.5	µg/mL
Xyzal [®]	Levo-Cetirizine	1.4	µg/mL

Table 6: Interferences of antihistamine drugs

Specificity

Samples from eight normal blood donors were double stained twice with anti-CCR3-PE and anti-CD3-AF647. The relative amount (mean) of CD3⁺ cells within the gated Basophil population was 3.9%.

Mean	3.9%
95th percentile	1.93 - 5.41%

Order code:

FK-HSBAT 100 tests



BÜHLMANN Laboratories AG
Baselstrasse 55
CH-4124 Schönenbuch/Basel
Switzerland

Phone +41 61 487 12 12
Fax orders +41 61 487 12 99
info@buhlmannlabs.ch
www.buhlmannlabs.ch