CAST® ELISA

Cellular Antigen Stimulation Test
ELISA

For research use only.
Not for use in diagnostic procedures.

EK-CAST-U 192 Tests
EK-CAST5-U 480 Tests

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Version A1

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ENGLISH

INTENDED USE

The BÜHLMANN CAST® ELISA is intended for the quantitative determination of sulfidoleukotrienes (sLT) produced by isolated leukocytes upon contact with specific antigens.
This product is for research use only. It is not intended for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

In the CAST® ELISA (ref 1), sedimented leukocytes from blood samples are simultaneously primed with Interleukin 3 (IL-3) and stimulated with allergens (ref 2). Basophilic cells among others generate the allergic mediator, sulfidoleukotriene LTC_4, and its metabolites LTD_4 and LTE_4. *De novo* formation of LTC_4 can be both, IgE dependent or non-IgE dependent. The latter event is usually described as pseudo-allergy. These freshly synthesized sLT are subsequently measured in an ELISA test (Enzyme Linked ImmunoSorbent Assay). The principle of the CAST® ELISA is covered by a patented technology (Patent No. US5487977).
The CAST® ELISA can be separated into three procedural parts:

1. Isolation of leukocytes

Dextran is added to blood sample in order to increase the blood viscosity. After 90 minutes at 18-28°C, the erythrocytes are sedimented, whereas leukocytes and thrombocytes stay in the plasma fraction. Subsequently, the blood supernatant is being carefully transferred into separate tubes and the leukocytes are sedimented in a brief centrifugation step. The plasma supernatant containing >90% of the thrombocytes is discarded and the leukocyte pellet is resuspended in the stimulation buffer containing IL-3.

2. Cell stimulation

Each cell is tested for the basal level release (= background) and for the release after stimulation with a specific allergen. The cells are stimulated during 40 minutes incubation at 37°C. Finally, the cells are centrifuged and the cell supernatant is either frozen for subsequent storage or immediately tested for sLT concentration in the ELISA.
As a positive control that proves the viability and functionality of the cells, a third blood sample is stimulated with an antibody (Ab) directed against the high affinity IgE receptor (FcεRI). Similar to an allergen, this antibody leads to the cross-linking of the Fcε receptor I and, therefore, to the stimulation of the cells. This anti-IgE receptor antibody binds to domain 1 on the α-subunit of the high affinity IgE receptor and is a non-inhibiting antibody. Thus, it will bind to the receptor irrespective of the receptor being free from or loaded with IgE.

3. Leukotriene determination

The ELISA is performed using precoated microtiter plates. 16 wells per assay are used for the standard curve and controls. Two wells per sample are used for the background, two wells per sample for the stimulation control and two wells for each allergen. Enzyme label (alkaline Phosphatase=aPase) and antibody are added to the cell supernatants as well as to the standards and controls and incubated. After a washing step, substrate solution (para-Nitrophenyl-Phosphate = pNPP) is added to each well and incubated. Finally, stop solution (2N NaOH) is added to each well and the color absorbance is measured at 405 nm in a microtiter plate reader.

REAGENTS SUPPLIED AND PREPARATION

Reagents for cell isolation and cell stimulation

Reagents	Quantity		Code	Reconstitution
	EK-CAST	EK-CAST5		
Dextran Solution w/o preservatives	1 vial 20 mL	2 vials 20 mL	B-CAST-DS	Ready to use
Stimulation Buffer with IL3; w/o preservatives	1 vial lyoph.	3 vials lyoph.	B-CAST-STB	add 50 mL of ultra-pure, apyrogenic water
Stimulation Control [6] anti-IgE Receptor Ab and fMLP; w/o preservatives	1 vial lyoph.	2 vials lyoph.	B-CAST-STCON	add 3.5 mL of ultra-pure, apyrogenic water

Table 1

ELISA Reagents

Reagents	Quantity		Code	Reconstitution
	EK-CAST	EK-CAST5		
Microtiter Plate precoated with polyclonal anti-murine IgG	2 plates: 12 x 8	5 plates: 12 x 8	B-CAST-MP	Wash 1x before use
Plate Sealer	6 pieces	15 pieces		
Wash Buffer Concentrate (20X) With preservatives	1 bottle 50 mL	3 bottles 50 mL	B-CAST-WB	Dilute with 950 ml of deionized water
ELISA Buffer With preservatives	1 bottle 30 mL	1 bottle 80 mL	B-CAST-EB B-CAST5-EB[1]	Ready to use
Calibrator[2] Leukotriene D_4 in a HSA buffer matrix	5 x 1 lyoph.	8 x 1 vial lyoph.	B-CAST-CA[3]	Add 1 mL of deionized water
Control Low / High[4] leukotriene D_4 in a HSA buffer matrix	5 x 2 lyoph.	8 x 2 vials lyoph.	B-CAST-CONSET[5]	Add 1 mL of deionized water
Blanking Reagent[2] Leukotriene D_4 in a buffer matrix	1 vial lyoph.	1 vial lyoph.	B-CAST-BR	Add 2 mL of deionized water
Enzyme Label Leukotriene D_4 conjugated to alkaline phosphatase, contains preservatives	2 vials lyoph.	3 vials lyoph.	B-CAST-ELS	Add 5.5 mL of ELISA Buffer
			B-CAST-EL[1]	Add 11 mL of ELISA Buffer
Antibody Monoclonal anti-sLT Ab in a buffer matrix with preservatives	1 vial 11 mL	1 vial 27.5 mL	B-CAST-AS B-CAST5-AS[1]	Ready to use
pNPP Substrate with stabilizing pellets	1 vial 42 mL	1 vial 105 mL	B-CAST-PNPP B-CAST5-PNPP[1]	Ready to use
Stop Solution 2 N NaOH	1 vial 11 mL	1 vial 27.5 mLl	B-CAST-NAOH B-CAST5-NAOH[1]	Ready to use **Corrosive agent**

Table 2

[1] Order code for EK-CAST5
[2] After reconstitution, the calibrator contains 3200 pg/mL of LTD_4 and the blanking reagent contains 32'000 pg/mL of LTD_4, respectively.
[3] Single calibrator reagent set: B-CAST-CA5 or -CA8 content: 5x or 8x B-CAST-CA.
[4] The controls contain lot-specific amount of LTD_4. Refer to the QC data sheet added to the kit for actual concentrations.
[5] Single control reagent set: B-CAST-CONSET5 or -CONSET8 content: 5x or 8x B-CAST-CONSET.
[6] Stimulation controls containing either anti-IgE receptor or fMLP may be ordered using the following order codes: B-CCR-STCON and B-CCR-FMLP, respectively.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Kit	
Store at 2-8°C except calibrators, blanking reagents and controls which must be stored at -20°C or below. Do not use past kit expiration date.	
Opened / reconstituted reagents	
Dextran Solution	Store at 2-8°C until expiration date printed on the label
Stimulation Buffer	Stable at -20°C for 6 months. Aliquot if repeated use is expected
Stimulation Control	Stable at 2-8°C for 2 months. For longer storage aliquot and freeze at -20°C for up to 6 months.
Microtiter Plate	Remove the unused strips from the holder. Return them to the foil pouch and reseal along the entire edge of zip-seal. Store for up to 6 months at 2-8°C.
Wash Buffer	Store for up to 2 months at 2-8°C
Calibrator	**Do not store**
Controls	
Blanking Reagent	Store at –20°C or below for up to 2 months. Aliquot if repeated use is expected
Enzyme Label	Store at 2-8°C for up to 2 months
ELISA Buffer	Store at 2-8°C until expiration date printed on the label
Antibody	
Substrate Solution	
Stop Solution	Store at 18-28°C or at 2-8°C until expiration date printed on the label

Table 3

ALLERGENS AND REAGENTS TO BE ORDERED ADDITIONALLY

Reagents	Quantity	Code
Allergens [1)]	1 vial	see CAST® Allergen list (www.buhlmannlabs.ch)
C5a recombinant human C5a	1 vial	BAG2-C5A-R
Stimulation Control Anti-IgE Receptor Antibody	1 vial	B-CCR-STCON
Stimulation Control fMLP	1 vial	B-CCR-FMLP

Table 4

[1)] Validated Allergens for the analysis in CAST® assays are offered by BÜHLMANN. Refer to the allergen list on the webpage to obtain the respective order codes (www.buhlmannlabs.ch).

- Protein allergens are shipped as concentrated liquids (1µl/vial) and must be diluted before use.
- Drug and chemical allergens are shipped lyophilized and must be reconstituted before use.

Refer to the BÜHLMANN allergen booklet and allergen data sheets available on the website www.buhlmannlabs.ch.

ALLERGEN REAGENTS FROM OTHER SOURCES

Allergens from other sources may be used in the CAST® ELISA with the following limitations:

- No matrix-bound allergens (solid or liquid phase).
- No allergen preparations containing leukotrienes.
- No allergen preparations containing cytotoxic compounds (stabilizers, preservatives) such as glycerol, phenol, sodium azide or merthiolate (thimerosal).

For the procedure to establish customer specific allergens for the CAST®-assays ask your local distributor or contact BÜHLMANN Laboratories AG.

MATERIALS REQUIRED BUT NOT PROVIDED

- Adjustable precision pipettes with disposable tips: 10-100 µL, 200-1000 µL, 1-5 mL.
- Adjustable dispenser with disposable tips: 25-1000 µL.
- Disposable polypropylene tubes for the cell separation.
- Disposable polypropylene or polystyrene tubes and tissue culture grade microtiter plates for the cell stimulation, respectively.
- Laminar flow for cell separation and stimulation (optional).
- Refrigerated centrifuge at 130 - 1000 x g.
- Ultrapure, apyrogenic water for the cell stimulation reagents.
- Water bath (or incubator) set at 37°C.
- 50 mL and 1000 mL cylinder for the buffer preparation.
- Microtiter plate washing/aspiration device.
- Distilled or deionized water.
- Blotting paper.
- Microtiter plate rotator.
- Microtiter plate reader (405 nm).

PRECAUTIONS

Safety precautions

- Human specimens may contain infectious agents e.g. Hepatitis B virus and HIV.
- Substrate and stop solution: The substrate solution contains para-nitrophenyl-phosphate (pNPP). The stop solution contains 2N sodium hydroxide. Each of those reagents is irritant to eyes, skin and mucous membranes. Wear suitable protective clothing, gloves and eye protection. After contact with eyes or skin, wash immediately with plenty of water
- Safety data sheet available for professional user on request.
- Unused solutions should be disposed of according to local state and federal regulations.

Technical Precautions

- The pNPP substrate solution is ready to use. Do not vortex or try to homogenize the stabilizing pellets prior to use.
- Recommended water quality for the CAST® ELISA:
  A.) Cell stimulation: The use of ultrapure, apyrogenic water for reconstituting cell stimulation reagents (stimulation buffer and stimulation control) is essential for good and reproducible leukocyte stimulation. The following sources of water may be used: Cell culture grade water, infusion grade water or ultrafiltrated, deionized double distilled water.
  B.) ELISA: All reagents must be reconstituted with ultrafiltered, deionized or double distilled water or the same water quality that is used for the cell stimulation reagents.

  Precautions to avoid allergen contamination during cell stimulation: Aeroallergens present in the laboratory may contaminate open blood samples and cell suspensions potentially causing elevated background or a falsely positive stimulation. Therefore, blood samples and cell stimulation tubes must be covered. Avoid dust mites, pollinating plants and open windows in the laboratory where the cell stimulation is performed. We recommend carrying out the cell preparation and stimulation steps in a laminar flow hood.

- Calibrator dilution: Reconstitute a new calibrator vial and prepare a fresh standard curve each time a new assay is performed. Reconstituted and diluted calibrators and controls are not stable and must be used in the ELISA without delay.
- Components must not be used after the expiry date printed on the labels. Do not mix different lots of reagents.
- Avoid contamination of reagents.
- Microwells cannot be re-used.
- It is important reading through the Instructions for Use prior to commencing the test. Reliable results will be obtained only if this Instruction for Use is followed accurately.
- Samples that are not properly handled may cause inaccurate results.

SPECIMEN COLLECTION AND STORAGE

Collect sufficient blood into EDTA venipuncture tubes. 200 µL of whole blood are needed per reaction tube. Calculate the amount of blood needed by the following table:

No. of allergens to be tested	Required blood (mL)
1-5	2.0
6-10	3.0
11-15	4.0
16-20	5.0

Table 5

Important:

- Perform the cell stimulation within 24 hours after blood collection.
- Blood sample should be refrigerated at 2-8°C.
- Do not centrifuge or freeze the blood.

ASSAY PROCEDURE

Preparation of the calibrators

In order to obtain the standard curve, serial dilutions of the calibrator are prepared as follows:

- Label three tubes S2 through S4 and pipet 300 µL of ELISA buffer into tubes S2 through S4.
- Pipet 100 µL of reconstituted calibrator (S1, 3200 pg/mL) into tube S2 and vortex.
- Transfer 100 µL from S2 to S3, vortex. Transfer 100 µL from S3 to S4, vortex.

The corresponding sLT concentrations will be:

S1: 3200 pg/mL
S2: 800 pg/mL
S3: 200 pg/mL
S4: 50 pg/mL
S0: Zero calibrator (ELISA buffer only)

Cell stimulation

The volumes needed in step 1. to 4. depend on the sample volume and must be determined according to the table below:

Blood Sample	Dextran Solution	Stimulation Buffer
1 mL	0.25 mL	1 mL
2 mL	0.50 mL	2 mL
3 mL	0.75 mL	3 mL

Table 6

The volumes in steps 1. to 8. are calculated from 2 mL blood sample volume tested for a single allergen as an example:

- Pipet 2 mL of blood into a polypropylene tube, add 0.5 mL of dextran solution and vortex gently at low speed.
- Incubate for 90 minutes at 18-28°C.
- Transfer the upper phase into a second tube and centrifuge it for 15 minutes at 130 x g and 18-28°C.
- Discard the supernatant and resuspend the cells in 2 mL of stimulation buffer. Proceed to step 5 without interruption.

  Note: The incubation in step 5. may be carried out in small polypropylene or polystyrene tubes and in non-activated polystyrene microtiter plates, respectively. The following procedure uses polypropylene tubes in step 5. as an example. If sufficient cell suspension from step 4. is available, the volumes in step 5. may be doubled. This allows aliquoting and freezing of the supernatants for eventually assaying the samples a second time.

- Label tubes for each sample: SB (sample background), SC (sample control), A1 (allergen 1), and so on.
- Pipet 50 µL of stimulation buffer (background) into the SB tube of each sample
- Pipet 50 µL of stimulation control into the SC tube of each sample.
- Pipet 50 µL of allergen into the corresponding sample tubes.
- Pipet 200 µL of each sample's cell suspension into the corresponding tubes.
- Vortex gently, cover the tubes and incubate in a water bath for 40 minutes at 37°C.
- Vortex to dissolve agglutinates. Centrifuge for 3 minutes at 1000 x g and 2-8°C. Refrigeration is recommended for obtaining a firm pellet and to prevent sLT degradation. Pipet carefully 2 x 100 µL of cell supernatant from each tube for use in step 3e. of the ELISA procedure.

  Important: Proceed immediately to the ELISA procedure or store the cell supernatants for up to 4 months at ≤-20°C.

ELISA procedure

1. Determine the number of capture antibody-coated microtiter plate strips required to test the desired number of samples and allergens plus 16 wells needed for running blanking reagent, calibrator and controls.

   Note: If you do not use all of the strips at once, remove the remaining strips from the holder, return them to the foil pouch, reseal along the entire edge of zip-seal, and store them refrigerated.

2. Wash the wells 1x using at least 300 µL of wash buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

3a. Pipet 100 µL of blanking reagent in duplicate into wells A1+A2.

3b. Pipet 100 µL of ELISA buffer (zero calibrator, S0) in duplicate into wells B1+B2.

3c. Pipet 100 µL of calibrator S4 (50 pg/mL) in duplicate into wells C1+C2.

Pipet 100 µL of calibrator S3 (200 pg/mL) in duplicate into wells D1+D2.

Pipet 100 µL of calibrator S2 (800 pg/mL) in duplicate into wells E1+E2.

Pipet 100 µL of calibrator S1 (3200 pg/mL) in duplicate into wells F1+F2.

3d. Pipet 100 µL of the low control in duplicate into wells G1+G2.

Pipet 100 µL of the high control in duplicate into wells H1+H2.

3e. Pipet 100 µL of each cell supernatant in duplicate into the subsequent wells.

4. Add 50 µL of Enzyme label to all wells.

   Add 50 µL of antibody to all wells.

   Cover the plate with a plate sealer, place the plate on a plate rotator set at 800-1000 rpm and incubate for 2 hours at 18-28°C.

   Note: Alternatively, incubate the plate for 16-20 hours at 2-8°C

5. Remove the plate sealer. Empty the wells and wash them three times using at least 300 µl of wash buffer per well. Strike the plate firmly onto blotting paper.

   Important: Allow the pNPP substrate solution to come to 18-28°C prior to use.

6. Add 200 µL of pNPP substrate solution to all wells.
7. Cover the plate with a plate sealer, place the plate on a plate rotator set at 800-1000 rpm, protect the plate from direct light and incubate for 30 minutes at 18-28°C.
8. Remove the plate sealer. Stop the reaction by adding 50 µL of stop solution to all wells. Mix shortly on the microtiter plate rotator.
9. Read the absorbance at 405 nm in a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

Glossary

Maximum binding (MB, S0): Technical maximum absorption of the ELISA used for the calculation of the corresponding percent bound (B/B_0) values.
Blank (NSB): Technical, non-specific absorption of the ELISA. The value is subtracted from calibrator, control and sample absorptions.

Standard curve

Record the absorbance at 405 nm for each calibrator, maximum binding (MB = S0) and blank (NSB) well.
Average the duplicate values, subtract the average of the blank wells (NSB) and record the averages (= corrected average absorbance).
Calculate the binding of each pair of calibrator wells as a percent of maximum binding, with the NSB-corrected MB absorbance taken as 100%:

$$B/B_0\ (\%) = \text{percent bound} = \frac{\text{net absorbance}}{\text{net MB absorbance}} \times 100$$

Plot the percent bound (vertical axis) versus the sLT concentration in picograms/mL (pg/mL) of the calibrators (horizontal axis) using a lin/log graph paper. Draw the best fitting curve or calculate the standard curve using a four parameter algorithm.

Samples and controls

Record the absorbance at 405 nm for each sample and control well.
Average the duplicate values, subtract the average of the blank wells and record the averages (= corrected average absorbance).
Calculate, as described above, the binding of each pair of sample and control wells as a percent of maximum binding, with the NSB-corrected MB absorbance taken as 100%.

Read the sLT concentration (pg/mL) from the horizontal axis by interpolation of the B/B_0 value of the samples and controls.

Note: If the absorbance of an unknown sample is greater than the highest calibrator, the cell supernatant should be diluted with ELISA buffer and assayed again according to the assay procedure. The additional dilution must be considered when calculating the final concentration of sLT present in the unknown sample.

Stimulation yield

Yield of Stimulation Control	=	sLT concentration (Stimulation Control)	–	sLT concentration (Background)
Yield of Allergen Stimulation	=	sLT concentration (Allergen Stimulation)	–	sLT concentration (Background)

For an example of typical results see and Figure 1. The results and standard curve are provided for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

ASSAY QUALITY CONTROL

In order to verify an accurate test performance, it is imperative to keep track of the following quality control parameters:

- OD reading of the assay blank: < 200 mOD
- Estimated dose at 50% binding (ED-50): 200-400 pg/mL sLT
- Level of sample background: < 200 pg/mL sLTs release
- Level of stimulation control: > 200 pg/mL sLTs release (after subtraction of the background).

QUALITY CONTROL

A thorough understanding of this package insert is necessary for the successful use of the product. Reliable results will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following this package insert.
Since there are no controls for sLT commercially available, we recommend using pools of cell supernatants containing different sLT levels for internal quality controls (cell supernatants are stable for up to two months at ≤–20°C). All controls should fall within established confidence limits. The confidence limits for the BÜHLMANN synthetic sLT controls are lot-specific and printed on the additional QC data sheet. The reproducibility of standard curve parameters and control values should be within established limits of laboratory acceptability. If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices ii) settings of the ELISA reader iii) expiration dates of reagents iv) storage and incubation conditions v) pNPP substrate solution should be colorless vi) purity of water.

PERFORMANCE CHARACTERISTICS

Kinetics of cell stimulation:

An example of the kinetics of sLT production is given in Figure 1. The kinetics may differ from one individual to another. However, a stable stimulation is reached after 40 minutes of incubation.

Intra-assay precision of the ELISA (Within-Run): 4.6%.

The intra-assay precision was calculated from the results of 24 pairs of values from each sample in a single run. The values are presented in pg/mL of sLT (Table 8).

Intra-assay precision of cell stimulation and ELISA combined: 11.3%

The statistics were calculated from the results of 10 replicates of cell stimulation from each sample in a single run. The values are presented as pg/mL of sLT (Table 9).

Inter-assay precision of the ELISA (Run-to-Run): 15.4%.

The inter-assay precision was calculated for each sample from the results of 20 pairs of values in 20 different runs. The values are presented as pg/mL of sLT (Table 10).

Analytical sensitivity of the ELISA: 19 pg/mL.

The minimal detectable dose of sLT was calculated to be 19 pg/mL by subtracting two SD from the mean of 20 zero calibrator replicates (ELISA buffer, S0) and intersecting this value with the standard curve obtained in the same run.

Dilution linearity: 114.6%.

Three samples after cell stimulation were assayed either undiluted or diluted with the ELISA Buffer. The values are presented in pg/mL of sLT (Table 11).

Spiking recovery: 99.5%.

Three unstimulated samples were assayed before and after spiking with varying amounts of sLT D_4. The values are presented in pg/mLF of slt (Table 12).

Specificity:

Crossreactivities of the monoclonal antibody used were determined at 50% binding (Table 13).

Standardization:

CAST® ELISA is calibrated with Leukotriene D4. $E_{280\ nm}$: 40.000 mol x L^{-1} x cm^{-1}.

APPENDIX I

TABLES

Typical data

	Conc. (pg/mL)	Absor-bance (OD)	B/B_0 (%)	Calc. Conc. (pg/mL)	CV Conc. (%)
Blank		0.146			
Blank		0.156			
Blank Avg.		**0.151**			**4.7**
Cal S0	0	1.327	100.0		
Cal S0	0	1.303	100.0		
Cal S0 Avg.	**0**	**1.315**	**100.0**		**1.3**
Cal S4	50	1.146	85.5	51	
Cal S4	50	1.155	86.3	49	
Cal S4 Avg.	**50**	**1.151**	**85.9**	**50**	**3.6**
Cal S3	200	0.829	58.2	195	
Cal S3	200	0.812	56.8	205	
Cal S3 Avg.	**200**	**0.821**	**57.5**	**200**	**3.9**
Cal S2	800	0.385	20.1	793	
Cal S2	800	0.381	19.8	807	
Cal S2 Avg.	**800**	**0.383**	**19.9**	**800**	**1.2**
Cal S1	3200	0.202	4.4	3455	
Cal S1	3200	0.213	5.3	2966	
Cal S1 Avg.	**3200**	**0.208**	**4.9**	**3200**	**10.8**
Ctrl. LOW		1.069		76	
Ctrl. LOW		1.050		83	
Ctrl. L. Avg.		**1.059**		**80**	**6.2**
Ctrl. HIGH		0.629		356	
Ctrl. HIGH		0.612		374	
Ctrl. H. Avg.		**0.620**		**365**	**3.5**
Background		1.185		40	
Background		1.153		49	
Backgr. Avg.		**1.169**		**45**	**14.3**
Stim. Control		0.295		1282	
Stim. Control		0.299		1248	
Stim. Ctrl Avg.		**0.297**		**1265**	**1.9**
Allergen		0.474		572	
Allergen		0.490		543	
Allergen Avg.		**0.482**		**557**	**7.6**

ED-20 = 797 pg/ml ED-50 = 262 pg/ml ED-80 = 71 pg/ml

Table 7

Standard curve

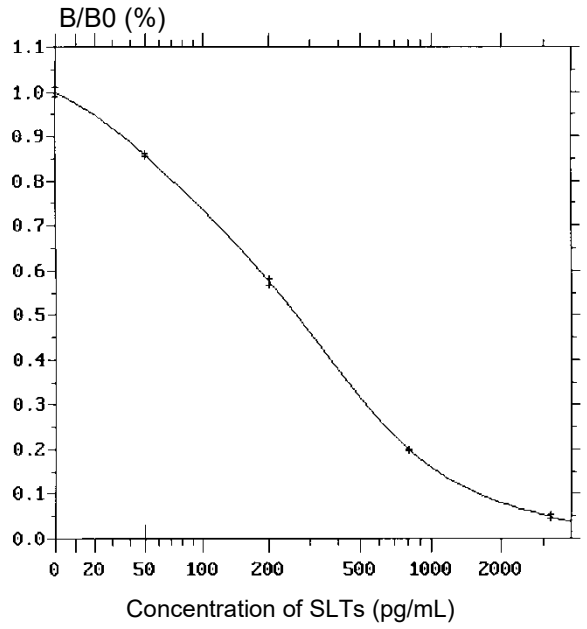


Figure 1

Kinetics of cell stimulation

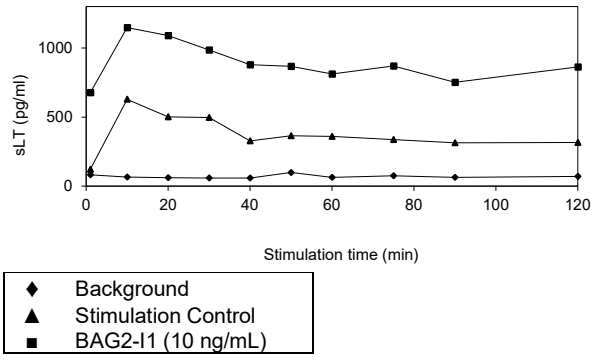


Figure 2

Intra-assay precision

Sample #	Mean	SD	% CV
1	54	3.9	7.3
2	221	9.6	4.4
3	467	13.9	3.0
4	592	22.3	3.8
5	1308	44.5	3.4
6	1752	94.9	5.4
Mean			4.6

Table 8

Intra-assay precision of cell stimulation and ELISA

Sample #		Mean	SD	% CV
7	Background	61	10	16
	Stimulation Control	1768	182	10
	BAG2-I1	582	31	5
8	Background	95	10	11
	Stimulation Control	873	81	9
	BAG2-I1	347	79	17
Mean				11.3

Table 9

Inter-assay precision ELISA

Sample #		Mean	SD	% CV
10	Background 1	90	16	18
11	Stimulation Control 2 (diluted)	494	61	12
10	BAG2-I1 1	1574	205	13
11	Stimulation Control 2	1604	332	21
10	Stimulation Control 1 (diluted)	2189	295	13
Mean				15.4

Table 10

APPENDIX I

TABLES

Dilution linearity

Sample #	Dil. Factor	Obs. [pg/mL]	Exp. [pg/mL]	Rec. O/E [%]
12	1:1	1337	--	--
	1:2	749	669	112.0
	1:4	380	334	113.7
	1:8	205	167	122.7
	1:16	91	84	108.9
13	1:1	1558	--	--
	1:2	874	779	112.2
	1:4	476	390	122.2
	1:8	252	195	129.4
	1:16	112	97	115.0
14	1:1	1421	--	--
	1:2	769	711	108.2
	1:4	408	355	114.8
	1:8	203	178	114.3
	1:16	90	89	101.3
Mean				114.6

Table 11

Spiking recovery

Sample #	Spiked with [pg/mL]	Obs. [pg/mL]	Exp. [pg/mL]	Rec. O/E [%]
15 Non-spiked: 17 pg/mL	50	75	67	111.9
	100	109	117	93.2
	200	204	217	94.0
	400	444	417	106.5
	800	801	817	98.0
	1600	1488	1617	92.0
	3200	2728	3217	84.8
16 non-spiked: 61 pg/mL	50	108	111	97.3
	100	182	161	113.0
	200	269	261	103.1
	400	442	461	95.9
	800	820	861	95.2
	1600	1588	1661	95.6
	3200	3086	3261	94.6
17 non-spiked: 40 pg/mL	50	102	90	113.3
	100	116	140	82.9
	200	257	240	107.1
	400	476	440	108.2
	800	862	840	102.6
	1600	1786	1640	108.9
	3200	2941	3240	90.8
Mean				99.5

Table 12

Specificity

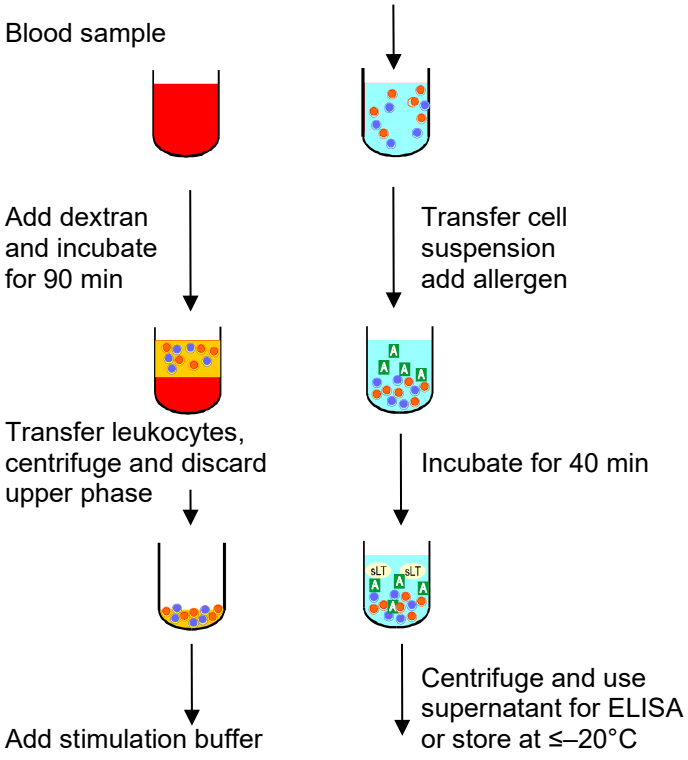
Leukotriene D_4	100.0%
Leukotriene B_4	1.1%
Leukotriene C_4	143.6%
5-Hydroperoxyeicosatetraenoic acid (HETE)	0.02%
Prostaglandine D_2	<0.01%
Prostaglandine E_2	<0.01%
Prostaglandine $F_{2\alpha}$	<0.01%
Thromboxane B_2	<0.01%
Leukotriene E_4	63.6%

Table 13

APPENDIX II

SHORT PROTOCOL

CAST® Cell Stimulation



TOTAL INCUBATION TIME FOR CELL STIMULATION: 130 MIN

CAST® ELISA

Precoated Microtiter Plate

wash 1 x

100 µL Standards, Controls or Cell Supernatants

add 50 µL Enzyme Label

add 50 µL Antibody

*incubate 2 hours (± 5 min) * at 18-28°C on a plate rotator*

wash 3 x

add 200 µL pNPP Substrate

incubate 30 min ± 5 min) at 18-28°C on a plate rotator

add 50 µL Stop Solution

→ Read absorbance at 405 nm (within 30 minutes)

TIME TO RESULT: 2.5 HOURS (FOR ELISA)

**alternative procedure: 16-20 hours at 2-8°C*

APPENDIX III

REFERENCES

1. Furukawa, K. *et a*l.: *Simplified sulfidoleukotriene ELISA using LTD4- conjugated phosphatase for the study of allergen-induced leukotriene generation by isolated mononuclear cells and diluted whole blood*. J Invest Allergol Clin Immunol **4**, 110-115 (1994)
2. Kurimoto, Y. *et al*.: *The effect of Interleukin 3 upon IgE-dependent and IgE-independent basophil degranulation and leukotriene generation.* Eur J Immunol **21**, 361-368 (1991).

APPENDIX IV

SYMBOLS

Symbol	Explanation
	Use By
REF	Catalogue Number
LOT	Batch Code
	Contains Sufficient For <n> Tests
	Consult Instructions for Use
	Temperature Limitation
	Upper Limit Of Temperature
SOLN DEX	Dextran Solution
BUF STIM	Stimulation Buffer
CONTROL STIM	Stimulation Control
MP	Microtiter Plate

Symbol	Explanation
BUF WASH 20X	Wash Buffer Concentrate (20x)
BUF ELISA	ELISA Buffer
CAL	Calibrator
CONTROL L	Low Control
CONTROL H	High Control
REAG BLANK	Blanking Reagent
EL	Enzyme Label
Ab	Antibody
SUBS PNPP	pNPP Substrate
SOLN STOP	Stop Solution