



# CAST® ELISA

Procedure

EK-CAST

A Commitment to Diagnostics

## Pre-Analytics

- Samples required** EDTA whole blood  
200 µl EDTA blood/sample collect at least 2 ml EDTA blood
- Sample storage** at 2-8°C up to 24 hours  
Do not freeze !
- Sample collection** Blood has to be drawn prior to skin testing or in vivo provocation
- Patient** Patient should discontinue a treatment with drugs such as antihistamines, corticosteroids or chromoglycic acid 3-7 days prior to sample collection

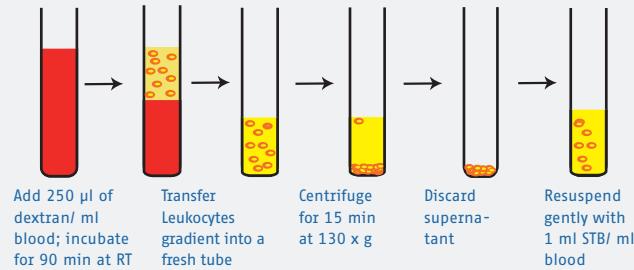
## Special Equipment

- Water bath (37°C)

## BÜHLMANN Allergens

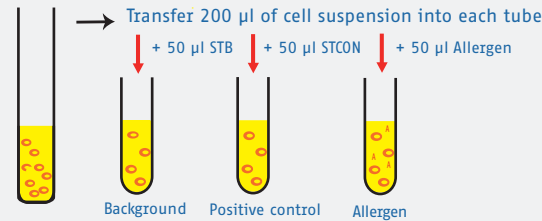
- Resuspend allergens with 250 µl Stimulation Buffer
- Up to 4 stimulations for each allergen vial
- In general, use freshly reconstituted allergens for the stimulation
- Reconstituted Bee&Wasp allergens (BAG2-I1 & BAG2I-3) are stable for one month stored at -20°C

## Isolation



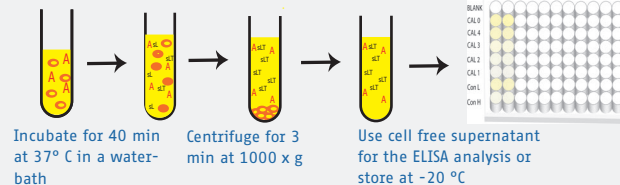
2h

## Stimulation Set-Up



20 min.

## Stimulation



40 min.

3 h

Time to Result:

## ELISA

All reagents must be adjusted to room temperature

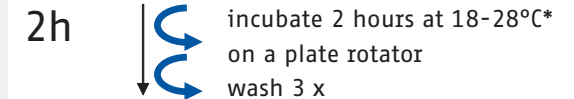
### Precoated Microtiter Plate



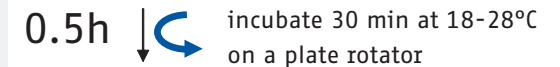
100 µl Standards, Controls or cell supernatants

add 50 µl Enzyme Label

add 50 µl Antibody



add 200 µl pNPP Substrate



add 50 µl Stop Solution

Read at 405 nm

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

~2.5 hours

~5.5 hours





# CAST<sup>®</sup> ELISA

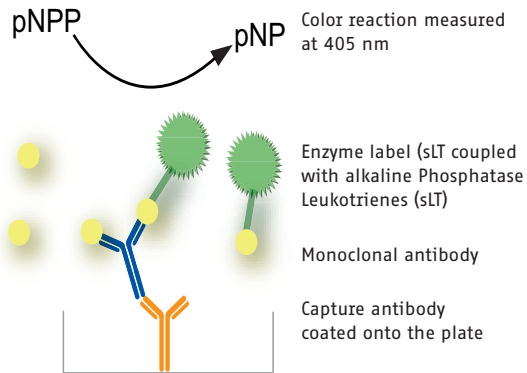
## Characteristics

## EK-CAST

A Commitment to Diagnostics

### Intended Use

Stimulation after contact with specific antigens and subsequent quantitative *in vitro* determination of de novo synthesised sulfidoleukotrienes samples by competitive immunoassay.



### Number of Patients to Test per Plate

with 1 Standard curve (<sup>1</sup>/<sub>1</sub> plate)

- 1 Allergen / 1 conc. 13 patients
- 2 Allergen / 1 conc. 10 patients
- 3 Allergen / 2 conc. 6 patients

with 2 Standard curves (<sup>1</sup>/<sub>2</sub> plate)

- 2 Allergen / 1 conc. 8 patients

### Assay Performance Data

#### Intra-assay precision

n=6; range: 54-1752 pg/ml  
24 duplicates; CV range: 3.0-7.3 %

#### Inter-assay precision

n=7; range: 90-2189 pg/ml  
20 repetitions; CV range: 12-21%

#### Dilution linearity

n=3; diluted 1:1 up to 1:16  
recovery range: 101-129%

#### Spiking recovery

n=3; spiked with Leukotrienes  
recovery range: 82.9-113%

#### Analytical sensitivity

n=20, measured in duplicates; mean + 2 SD

#### Standard range

50-3200 pg/ml

### Data Calculation for Stimulation Yield

Sample	Stimulation sLT [pg/ml]	Yield sLT* [pg/ml]	
Patient background	55		
Stimulation control	1800	1745	
Bee allergen BAG2-I1	1000	945	POS
Wasp allergen BAG2-I3	200	145	NEG

\* Yield sLT= Allergen [pg/ml] - Background [pg/ml]

4.6 %

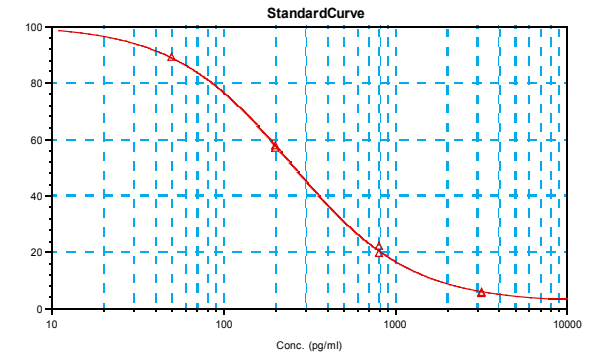
15.4 %

114 %

99.5 %

19 pg/ml

### Typical Standard Curve (4 Parameter Logistics)



### Cut off

- inhalant/ food allergens 200 pg/ml
- „chemical“ allergens Allergen specific\*
- bee & wasp (clinical cut-off) 270 pg/ml

\* see allergen booklet added to the kit

CAST<sup>®</sup> is a registered trademark of BÜHLMANN in many countries.

#### Ordering code:

EK-CAST 192 wells  
EK-CAST5 480 wells

CE-marked products



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