**CAST® ELISA**

**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**

- Add 250 µl of dextran/ml blood; incubate for 90 min at RT
- Transfer Leukocytes gradient into a fresh tube
- Centrifuge for 15 min at 130 x g
- Discard supernatant
- Resuspend gently with 1 ml STB/ml blood

**Stimulation Set-Up**

- Transfer 200 µl of cell suspension into each tube
- Incubate 2 hours at 18-28°C on a plate rotator
- Wash 1x

**Stimulation**

- Incubate for 40 min at 37°C in a waterbath
- Centrifuge for 3 min at 1000 x g
- Incubate for 40 min at 37°C in a waterbath
- Mix cell free supernatant for the ELISA analysis or store at -20°C

**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
  - incubate 2 hours at 18-28°C on a plate rotator
  - wash 3 x
- 0.5h incubate 30 min at 18-28°C on a plate rotator
- Add 250 µl of dextran/ml blood; incubate for 90 min at RT
- Transfer Leukocytes gradient into a fresh tube
- Centrifuge for 15 min at 130 x g
- Discard supernatant
- Resuspend gently with 1 ml STB/ml blood

**Time to Result:**

- ~2.5 hours
- ~5.5 hours

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**Supplied by:**

**alpha laboratories**

**Address:**

Alpha Laboratories Ltd
40 Parham Drive
Hampshire SO50 4NJ
UK

**Tel:** +44 23 8048 3000

**Email:** sales@alphalabs.co.uk

**Website:** www.alphalabs.co.uk
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.

**Enzyme label** (sLT coupled with alkaline Phosphatase)

**Monoclonal antibody**

**Capture antibody** coated onto the plate

**Characteristics**

**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
- 19 pg/ml

**Standard range**
- 50-3200 pg/ml

**Data Calculation for Stimulation Yield**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stimulation sLT [pg/ml]</th>
<th>Yield sLT* [pg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient background</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Stimulation control</td>
<td>1800</td>
<td>1745</td>
</tr>
<tr>
<td>Bee allergen BAG2-I1</td>
<td>1000</td>
<td>945 POS</td>
</tr>
<tr>
<td>Wasp allergen BAG2-I3</td>
<td>200</td>
<td>145 NEG</td>
</tr>
</tbody>
</table>

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

**Typical Standard Curve (4 Parameter Logistics)**

**Cut off**

- **Inhalant/food allergens**: 200 pg/ml
- **"Chemical" allergens**: 270 pg/ml

**BÜHLMANN**

CAST® is a registered trademark of BÜHLMANN in many countries.

**Ordering code:**
- EK-CAST 192 wells
- EK-CAST5 480 wells

*marked products

**Supplied by:**

alpha laboratories

 supplying quality to science

Alpha Laboratories Ltd
40 Parham Drive
Hampshire SO59 4NJ  UK
Tel: +44 23 8048 3000
Email: sales@alphalabs.co.uk
Web: www.alphalabs.co.uk