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 Leucocytes 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 for 40 min at 37°C in a water-bath
- Centrifuge for 3 min at 1000 x g
- Rinse cell free supernatant for the ELISA analysis or store at -20°C

Stimulation
- Incubate for 40 min at 37°C
- Centrifuge for 15 min at 130 x g
- Discard supernatant

ELISA

Precoated Microtiter Plate
- wash 1x
- 100 µl Standards, Controls or cell supernatants
- add 50 µl Enzyme Label
- add 50 µl Antibody
- incubate 2 hours at 18-28°C*
- wash 3 x
- add 200 µl pNPP Substrate
- incubate 30 min at 18-28°C on a plate rotator
- add 50 µl Stop Solution
- Read at 405 nm

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

Time to Result: ~5.5 hours
CAST® ELISA

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

Characteristics

Assay Performance Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay precision</td>
<td>4.6%</td>
</tr>
<tr>
<td>n=6; range: 54-1752 pg/ml</td>
<td></td>
</tr>
<tr>
<td>24 duplicates; CV range: 3.0-7.3%</td>
<td></td>
</tr>
<tr>
<td>Inter-assay precision</td>
<td>15.4%</td>
</tr>
<tr>
<td>n=7; range: 90-2189 pg/ml</td>
<td></td>
</tr>
<tr>
<td>20 repetitions; CV range: 12-21%</td>
<td></td>
</tr>
<tr>
<td>Dilution linearity</td>
<td>114%</td>
</tr>
<tr>
<td>n=3; diluted 1:1 up to 1:16</td>
<td></td>
</tr>
<tr>
<td>recovery range: 101-129%</td>
<td></td>
</tr>
<tr>
<td>Spiking recovery</td>
<td>99.5%</td>
</tr>
<tr>
<td>n=3; spiked with Leukotrienes</td>
<td></td>
</tr>
<tr>
<td>recovery range: 82.9-113%</td>
<td></td>
</tr>
<tr>
<td>Analytical sensitivity</td>
<td>19 pg/ml</td>
</tr>
<tr>
<td>n=20, measured in duplicates; mean + 2 SD</td>
<td></td>
</tr>
<tr>
<td>Standard range</td>
<td>50-3200 pg/ml</td>
</tr>
</tbody>
</table>

Typical Standard Curve (4 Parameter Logistics)

Cut off
- inhalant/ food allergens 200 pg/ml
- "chemical" allergens 100 pg/ml
- Bee allergen (clinical cut-off) 270 pg/ml
- Wasp allergen 200 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]

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CAST* is a marked product.