CAST® ELISA
Cellular Antigen Stimulation Test
ELISA

Application for horse allergy testing
Supplementary to instruction for use

EK-CAST 192 tests

For Research Use Only (RUO)

Revision date: 2011-05-02
This application of BÜHLMANN CAST® ELISA is designed for the quantitative determination of sulfidoleukotrienes (sLT) produced by isolated horse leukocytes upon contact with specific allergens.

**MATERIALS REQUIRED BUT NOT PROVIDED**
ACD-B Vacuette 9 ml (Greiner bio-one yellow cap with black ring, #455094) or special S-Monovette CPDA 8.5 ml (Sarstedt #01.1610.001).

**ALLERGENS AND REAGENTS REQUIRED AND SUPPLIED UPON REQUEST**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Quantity</th>
<th>Code</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culiculides Allergen lyophilized</td>
<td>1 vial</td>
<td>BAG2-CUL</td>
<td>lyoph.</td>
</tr>
<tr>
<td>Stimulation Control</td>
<td>1 vial; 1.0 ml</td>
<td>B-EQCAST-CONA</td>
<td>Ready to use</td>
</tr>
</tbody>
</table>

**ALLERGENS AND REAGENTS SUPPLIED BUT NOT NEEDED**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Quantity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran Solution w/o preservatives</td>
<td>1 vial; 20 ml</td>
<td></td>
</tr>
<tr>
<td>Stimulation Control human anti-IgE Receptor Ab w/o preservatives</td>
<td>1 vial lyoph.</td>
<td></td>
</tr>
</tbody>
</table>

**IMPORTANT:**
Collect sufficient blood into tubes. Determine the minimum amount of blood needed according to the following table:

<table>
<thead>
<tr>
<th>No. of allergen concentrations to be tested</th>
<th>Required sample volume [ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>7.5</td>
</tr>
<tr>
<td>6-10</td>
<td>15.0</td>
</tr>
</tbody>
</table>

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

**INTERPRETATION OF RESULTS**

**INTERPRETATION OF STIMULATION CONTROL**
In allergic as well as in non-allergic individuals, a net stimulation control yield (after subtraction of background) of at least 200 pg/ml should be expected using the Stimulation Control. The Stimulation Control represents leukotrienes production due to cross-linking with concanavalin A on basophils.

**INTERPRETATION OF ALLERGEN STIMULATION**
For Culiculides Allergen, we propose that samples with a net stimulation yield higher than 350 pg/ml sLT should be regarded as positive for the allergen tested.

**LIMITATIONS**
A negative CAST® ELISA result for a Culiculides allergen can not exclude the potential occurrence of a (even severe) clinical reaction in a patient. Patients with a history of adverse reactions to a drug allergen with a negative CAST® ELISA result should therefore be followed up with an additional method, such as an in vivo provocation or skin prick test (where appropriate), before any drug is administered.
**CAST® Cell Stimulation**

- Horse sample
- Let blood sediment for 30 min
- Transfer cell suspension
- Add allergen
- Transfer leukocytes, centrifuge for 15 min and discard upper phase for 40 min
- Add stimulation buffer

**TOTAL INCUBATION TIME FOR CELL STIMULATION:** 78 Min

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**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
- Centrifuge and use supernatant for ELISA
  - Add 50 µl Stop Solution

**TIME TO RESULT:** 2.5 Hours (for ELISA)