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)

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BÜHLMANN LABORATORIES AG
Baselstrasse 55
CH - 4124 Schönenbuch, Switzerland
Tel.: +41 61 487 1212
Fax: +41 61 487 1234
info@buhlmannlabs.ch

ENGLISH

INTENDED USE

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

Reagents	Quantity	Code	Comments
Culicuides Allergen [1)] lyophilized	1 vial	BAG2-CUL	lyoph.
Stimulation Control Concanavalin A	1 vial; 1.0 ml	B-EQCAST-CONA	Ready to use

Table 1

ALLERGENS AND REAGENTS SUPPLIED BUT NOT NEEDED

Reagents	Quantity
Dextran Solution* [1]w/o preservatives	1 vial; 20 ml
Stimulation Control human anti-IgE Receptor Ab w/o preservatives	1 vial lyoph.

Table 2

IMPORTANT:

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

No. of No. of allergen concentrations to be tested	Required sample volume [ml]
1-5	7.5
6-10	15.0

Table 3

IMPORTANT:

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

ASSAY PROCEDURE

CELL ISOLATION AND STIMULATION

1. Let 7.5 / 15 ml horse ACD-blood sediment for 30 min. at 18-28℃.
2. Transfer leucocytes (upper phase) into a fresh tube.
3. Centrifuge leucocytes for 15 minutes at 130 x g and 18-28℃.
4. Discard the supernatant and resuspend the cells in 1 ml of Stimulation Buffer. *Proceed to step 5 without interruption*.

   Notes: The incubation in step 5. may be carried out in small polypropylene or polystyrene tubes and in non-activated polystyrene microtiter plates, respectively.

5a. Label tubes for each sample: SB (sample background), SC (sample control), A1 (allergen 1), and so on.

5b. Pipet 50 µl of *Stimulation Buffer (background*) into the PB tube of each sample.

5c. Pipet 50 µl of *Stimulation Control* (Concanavalin A) into the PC tube of each sample.

5d. Pipet 50 µl of *Culicuides Allergen* into the corresponding sample tubes.

6. Pipet 200 µl of each sample's cell suspension into the corresponding tubes.
7. Vortex gently, cover the tubes and incubate in a water bath for 40 minutes at 37℃.
8. Vortex to dissolve agglutinates. Centrifuge for 3 minutes at 500 x g at 2-8℃.
9. Pipet carefully 2 x 100 µl cell free supernatant from each tube and and measure leucotrienes immediately. (Refer to in step 3e. of the ELISA procedure).

   Important: Proceed immediately to the ELISA or store the cell supernatants for up to 4 months at ≤20℃.

ELISA

The ELISA procedure is described in detail in the instruction for use delivered with this kit (CAST® ELISA, Code:EK-CAST)

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 Culicuides 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.

NOTES

CAST® Cell Stimulation

Horse sample

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let blood sediment for 30 min

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Transfer leukocytes, centrifuge for 15 min and discard upper phase for 40 min

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Add stimulation buffer

Transfer cell suspension add allergen

Incubate

Centrifuge and use supernatant for ELISA or store at –20°C

TOTAL INCUBATION TIME FOR CELL STIMULATION: 78 MIN

CAST® ELISA

Precoated Microtiter Plate

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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

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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)