



CAST® ELISA

Application for horse allergy testing

A Commitment to Diagnostics

Pre-Analytics

- Samples required** ACD blood (citrate)
7.5 ml blood/sample
- 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.

Special Equipment

- water bath (37°C)

Allergens

- Resuspend the allergen with 250 µl Stimulation Buffer. Up to 4 stimulations for each vial.
- Store unopened vials at 2-8°C until exp. date.
- Use freshly reconstituted allergen.
- Culicoides allergen is available upon request.

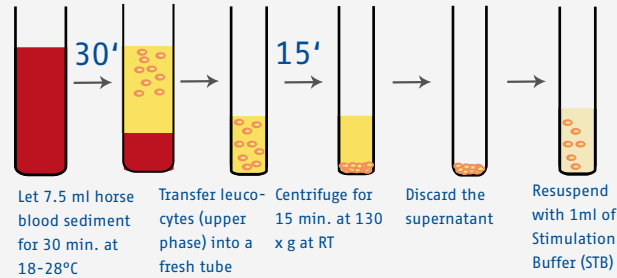
Reagents required but not delivered

Reagents	EK-CAST	Comments
Stimulation Control; (Concanavalin A)	1 x 1 ml	Ready to use; available upon request; Code: B-EQCAST-CONA
Culicoides Allergen	1 x lyoph.	add 250 µl H ₂ O BAG2-CUL

Reagents delivered but not required

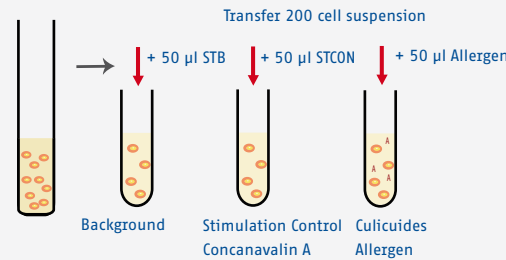
Dextran-Solution	1 x 20 ml
Stimulation Control; (human anti-IgE receptor ab)	1 x lyoph.

Isolation



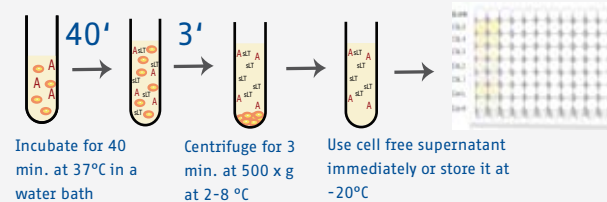
45 Min.

Stimulation Set-Up



20 Min.

Stimulation



43 Min.

~ 2 h

Time to Result: ~4.5 hours

ELISA

All reagents must be adjusted to room temperature

Precoated Microtiter Plate

↓ wash 1x

100 µl Standards, Controls or cell supernatants

+ add 50 µl Enzyme Label

+ add 50 µl Antibody

2h ↓ incubate 2 hours at 18-28°C on a plate rotator
wash 3 x

add 200 µl TMB Substrate

0.5h ↓ incubate 15 min at 18-28°C on a plate rotator

add 50 µl Stop Solution

→ Read at 405 nm

~2.5 hours





CAST[®] ELISA

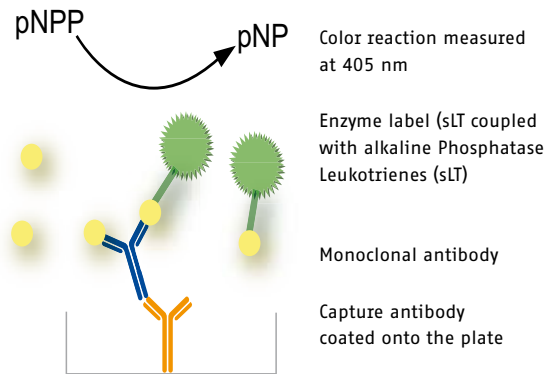
Characteristics

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 Samples per Test

- with 1 Standard curve (¹/₁ plate)
 - 1 Allergen/ 1 conc. 13 samples
- with 2 Standard curves (¹/₂ plate)
 - 1 Allergen/ 1 conc. 10 samples

Assay Performance Data

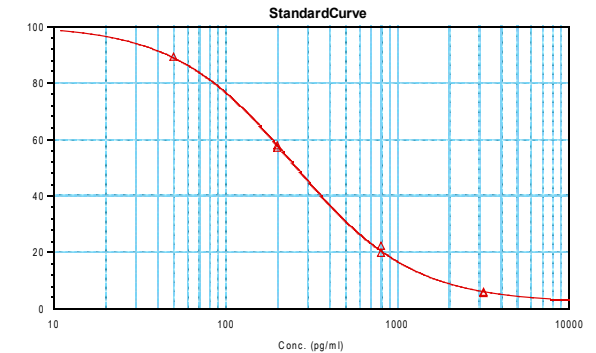
- Intra-assay precision** 4.6 %
n=6; range: 54-1752 pg/ml
24 duplicates; CV range: 3.0-7.3 %
- Inter-assay precision** 15.4 %
n=7; range: 90-2189 pg/ml
20 repetitions; CV range: 13-21%
- Dilution linearity** 114 %
n=3; diluted 1:1 up to 1:16
recovery range: 101-129%
- Spiking recovery** 99.5 %
n=3; spiked with Leukotrienes
recovery range: 82.9-108.9%
- Analytical sensitivity** 19 pg/ml
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]	
Sample background	45		
Stimulation control	1265	1220	
Culicoides Allergen BAG2-CUL	557	512	POS

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

Typical Standard Curve (4 Parameter Logistics)



Cut off

Culicoides Allergen 350 pg/ml

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



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