

Canine INgezim PLEX Food Sensitization kit

R. 15.CFA.K.61

Canine INgezim® PLEX Food Sensitization kit is an ELISA- microarray immunoassay designed to simultaneously measure the semi-quantitative level of 22 food allergen-specific IgE in canine serum. The kit is intended to offer veterinarian clinicians a support for designing an elimination diet trial in selected patients to confirm or rule out food allergy.

KIT FEATURES

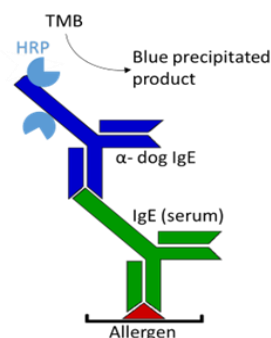
APPLICATION

The kit is intended for the determination of allergen-specific IgE levels that may have clinical significance in dogs previously diagnosed with canine topical dermatitis. It is exclusively valid for sera from dogs suffering this pathology.

TECHNICAL BASE

The Canine INgezim® PLEX Food Sensitization tests are solid-phase immunoassays in which allergen extracts are deposited in duplicate spots forming a microarray onto microplate wells. The immunoassay is performed in an indirect ELISA-microarray format, in which the allergen-specific IgEs present in a sensitized dog serum will bind to the corresponding allergen spots. The specific allergen-IgE binding is detected by the HRP-conjugated anti-dog IgE antibody. After a second washing step, the binding is developed by a colorimetric peroxidase substrate (TMB) which is converted to an insoluble, coloured product that precipitates specifically onto the allergen-specific IgE reactive spots. The enzyme-substrate reaction is stopped by washing with ddH₂O to remove TMB substrate. The amount of precipitate is proportional to the level of allergen-specific IgE in the patient sample.

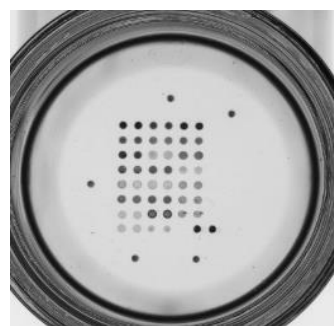
The assayed microarrays can be analyzed with our Hailstorm analyzer or by any commercial colorimetric reader compatible with 96-well microplates.



ALLERGEN PANEL

The microarray panel comprises 22 extracts (8 meat, 2 fish, 9 vegetables, egg, milk and yeast) deposited in duplicated spots on each microplate well. The microarray also features control spots to check the conjugate (dog IgE spots) and the serum sample (anti-dog IgE mAb spots) functionality and five positional marker spots for the Hailstorm's software image analysis.

Meat	Vegetables	Others
Beef	Soybean	Egg
Turkey	Corn	Milk
Pork	Wheat	Yeast
Chicken	Oat	Whiting
Lamb	Barley	Salmon
Duck	Rice	Dog IgE (positional markers)
Rabbit	Beet	Dog IgE (assay control spots)
Deer	Carrot	α-dog IgE mAb (canine serum control)
	Potato	



RESULT INTERPRETATION

Allergen-specific IgE reactivity is reported as signal intensity (SI) value in arbitrary units (AU). Spot intensity is measured as the grey signal intensity median of the spot pixels minus the grey signal intensity median of the background spots. Allergen-specific IgE reactivity (SI) is calculated as the average of the signal intensity of the spot duplicates.

The test determines reactive and non-reactive specific IgE to each of the 22 allergens included in the panel, based on a cutoff point established to discriminate between allergen-specific levels with or without potential clinical relevance. The microarray also includes both duplicate spots for evaluating the assay performance and the functionality of the serum.

As there is no evidence that the level of allergen-specific IgE correlates with the severity of clinical disease, the inclusion of a food allergen in an elimination diet is based on positive or negative results.

ASSAY VALIDATION

VALIDATION DATA

The assay was validated by comparison with Food 16 Screen IgE (Dog/Hund) ELISA (Immunolab) using 37 sensitized canine sera against at least one food extract and 116 negative canine sera. The comparison is based on positive and negative correlation and not on correlation of qualitative results due to the lack of gold standard test, differences in immunoassay format and units of measurement. The overall positive and negative correlation is 71% (27/38) and 91% (117/128), respectively.

Accuracy in inter-batch qualitative results (positive / negative): 97%

CV intra-batch: 2% - 23%

CV inter-batch: 8% - 25

HAILSTORM ANALYZER AND STORM 3.0 SOFTWARE DESCRIPTIONS

HAILSTORM ANALYZER

The Hailstorm (GSD US) is an automatic device of the bolt family which supports various technologies (ELISA, CLIA and ELISA microarray) and test formats (96 well MTP and Mono Test Strips).

The Hailstorm is a standalone two-plate device that process INgezim® PLEX microarray kits, providing analysis and interpretation of the results as specified in the kit instructions for use. The samples and reagents are processed in wells on a 96 well microtiter plate according to the assay protocol defined in the Test File. In this format, samples and reagent bottles are loaded into a reagent rack on the instrument. Hailstorm calculates the required volume to perform the assay depending on the sample number.

The Hailstorm allows to select the number and the position of the samples in the 96-well microplate. Ninety-six sera sample (20 µl serum per assay) analysis takes approximately 4.5 hours. The assay procedure is followed by an automatic scanning, greyscale image acquisition and analysis and data processing. Allergen-specific IgE reactivity is expressed as signal intensity (SI) values in arbitrary units (AU). In addition, performs and analyzes the microarray immunoassay providing a report of the patient's sensitization profile.

The relevant system components of the Hailstorm for microarray processing are:

- A high-resolution camera/lens to capture microarrays images on the microplate wells. It provides 10 bit gray scale images (1024 gray levels) which are 1.3 MegaPixels and 5.3 µm of pixel size.
- A LED illumination unit consisting of a circular array of 14 white LEDs, which can be moved to any microplate well position.
- A removable Diffusion Plate to create a uniformly illuminated background.



STORM 3.0 SOFTWARE

The Storm 3.0 software captures and analyzes microarray images from Ingezim PLEX microarray kits:

- Execute Microarray test files and display and report results as well as subsets of results.
- Store microarray images and data as well as backup and purge microarray data.

KIT COMPOSITION

- Allergen microarray microplate
- Vial with CCD inhibitor solution
- Bottle with HRP-Conjugate
- Bottle with Washing Solution
- Bottle with serum dilution buffer
- Bottle with substrate for HRP (TMB)
- Bottle with stop solution

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