



**Polycheck
EQUUS**

Polycheck Allergy NF Veterinary-Panel (Horse)

Test principle

This new *in vitro* test for the determination of allergen-specific IgE in serum is based on an immunoassay principle, using coated allergens and monoclonal antibody directed against horse-IgE.

During the first incubation step with serum the allergen-specific IgE molecules bind to the corresponding allergens. Subsequent to a washing step, the antibody detect the bound IgE. A second specific antibody mark the bound monoclonal antibody and react in a further incubation step with streptavidin-alkaline-phosphatase conjugates. After washing and addition of 5-bromo-4-chloro-3-indolyl-1-phosphate/nitro blue tetrazolium (BCIP/NBT) the enzymes develop a coloured precipitate. The amount of precipitate is directly proportional to the concentration of specific IgE in the applied serum, which results in a more or less intense colouring of each individual allergen.

The convincing advantages of our system are the possible determination of 20 allergens in only one assay run, an individual standard curve, a rather high sensitivity due to a high amount of allergens coated onto the membrane in form of narrow lines and the calculation of results by an image processing software.

Reagents provided in one test kit

All unopened reagents are stable if stored at 2-8°C until expiration date (max. 1 year).

- Allergy Cassettes (24 Cassettes): Allergy Cassettes containing one membrane coated with 20 allergens and 5 standards.
- Starter Solution (1 vial, 7 ml, ready to use): Buffered protein solution.
- 1.Detection antibody (1 vial, 7 ml, ready to use): Monoclonal antibodies against horse-IgE in a protein solution.
- 2.Detection antibody (1 vial, 7 ml, ready to use): Antibody against mouse-IgG in a protein solution.
- Streptavidin conjugate (1 vial, 7 ml, ready to use): Streptavidin conjugated to alkaline phosphatase.
- BCIP/NBT Substrate solution (1 vial, 7 ml, ready to use):
5-bromo-4-chloro-3-indolyl-1-phosphate/nitro blue tetrazolium as substrate for alkaline phosphatase.
- Wash buffer (1 poche): PBS based buffer + additives, which is used for washing the membrane.

Materials required

- Pipet (200- 1000 µl)
- Wipp-shaker (30 rpm)
- Computer with scanner and special software (provided by manufacturer)

Assay procedure

Common advice:

- Bring all reagents to room temperature.
- Proceed without interruption.
- Do not allow the membrane of the test Cassettes to dry during the assay.
- Pipett reagents onto the gap not covered by the membrane.

1. Overlay the Cassettes with wash solution, than tap on adsorbent paper.
2. To start add **250 µl starter solution** onto the gap, after **5 minutes** discard the fluid and tap the chip on filter paper.
3. Add **250 µl serum**. Incubate for **90 minutes** at room temperature under constant shaking at 30 rpm.
Tip the serum and wash the membrane three times with 1 ml wash solution.
4. Add **250 µl wash solution** and incubate for **5 minutes** at room temperature under constant shaking at 30 rpm.
Wash membrane as described in step 3.
5. Add **250 µl detection antibody 1** Incubate for **90 minutes** at room temperature under constant shaking at 30 rpm.
Wash membrane as described in step 3.
6. Add **250 µl detection antibody 2** Incubate for **60 minutes** at room temperature under constant shaking at 30 rpm.
Wash membrane as described in step 3.
7. Add **250 µl streptavidin conjugate** and incubate for **20 minutes** at room temperature under constant shaking at 30 rpm.
Wash membrane as described in step 3.
- 8.. Add **250 µl substrate solution (BCIP/NBT)** and incubate **in the dark** for **20 minutes** at room temperature under constant shaking at 30 rpm.
Wash membrane as described in step 3.
(total time: 290min)

Let the membrane dry sufficiently and analyse the Cassette using Biocheck Image Software.

Calculation of results

A computer with scanner and a special software serves to calculate the results:

Placed on top of a flatbed scanner the Cassettes are read out and documented. A special software programme analyzes and calculates the image data. In comparison to the standard curve the amount of allergen-specific IgE for each allergen is given as relative kilo units per litre (kU/l) (see Table 1)

Tab. 1 The 5 levels of allergic reactions

Concentration of IgE (kU/l)	level
< 1.0	0
1.0 – 2.0	1
2.0 – 20	2
> 20	3,4

Allergy panels

The veterinary Polycheck allergy panel contains: 15 single allergens and five mix (pollens: grass, trees, weeds; house dust mites; storage mites, moulds; insects).



Tyrophagus
Acarus siro
Stable fly (Stomoxys cal.)
Black fly (Simulium eq.)
Mosquito
Biting midges (Culicoides nub.)
Horse fly (Tabanus)
Rape
Mugwort
English plantain
6-Grassmix
Rye, common
Platane/Willow/Poplar
Birch/Alder/Hazel
Ambrosia (Ragweed)
Micropolyspora faeni/Thermoactinomyces
Asp.fumigatus/Pen.notatum
Lepidoglyphus destructor
D.pteronyssinus
D.farinae

Standard 1 –5