HESKA

TECHNICAL NOTE

CANINE TOTAL IgE TEST

The Heska's Canine total IgE test is a quantitative assay to measure total IgE in serum samples. The test is designed to provide high accuracy and specificity by using a combination of monoclonal antibody as IgE capturing antibody and the Fc-epsilon receptor as detecting IgE reagent.

The OD obtained with the sample is compared to the OD obtained in a purified canine IgE standard curve.

Principle

- ELISA (sandwich) test.
    - Monoclonal anti-Canine IgE coated on solid phase.
    - IgE detecting reagent: Fc-epsilon receptor.

Technical aspects

- A positive control and a blank must be included in each testing session.
- It is recommended to run at least the test in duplicate.
- The total IgE results are obtained in µg/ml.
    - IgE concentrations are read in the standard curve provided.

Sample preparation

The serum sample is used at a starting dilution of 1/3000 in sample diluent buffer.

Canine-Total IgE Kit content

- 1 plate (96well) of anti-IgE coated wells (plates: breakable type) – storage 4°C
- Biotinylated Fc-epsilon receptor: 200µl – **storage -20°C**
- Streptavidin Alkaline Phosphatase: 200 µl – storage 4°C
- Chromogen (pNPP): 15mls (ready to use) – storage 4°C
- IgE positive control: 1.5ml (ready to use) – storage 4°C
- 1 standard curve (graphic) for result calculation

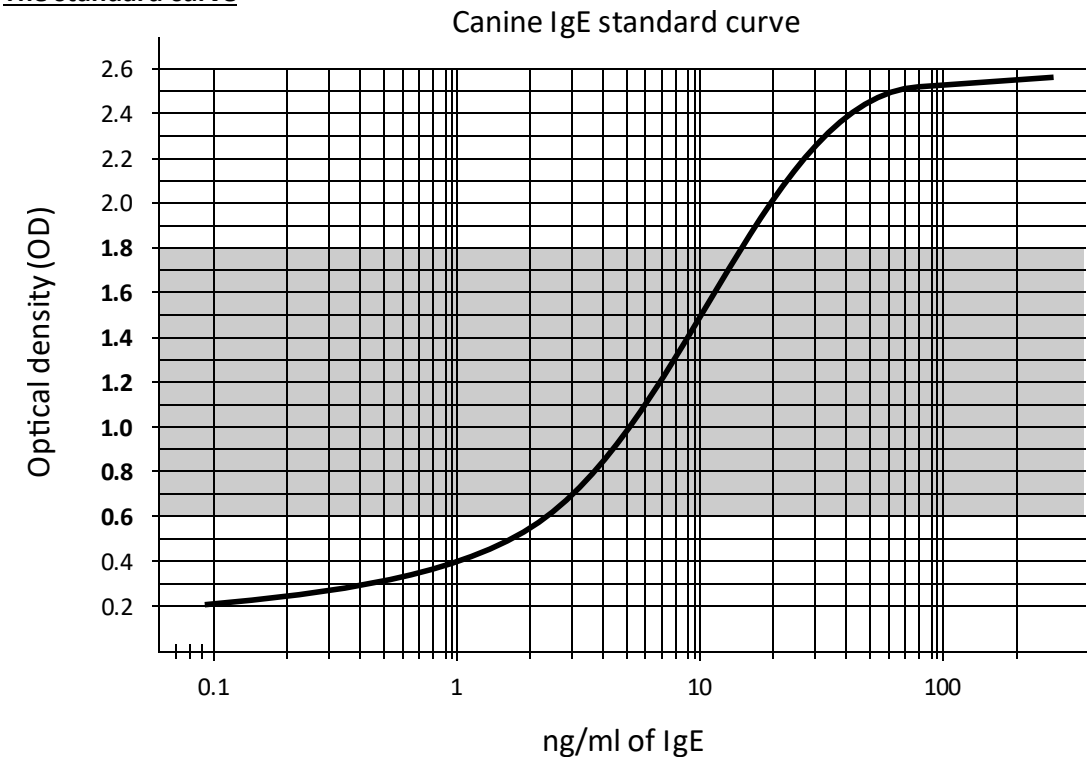
The result reading

Typically, the 1/3000 sample dilution, provides an expected OD between 0.6 and 1.8 OD in most cases which corresponds to the highest slope of the standard curve. It is in this range where the measures have their maximum accuracy. It is therefore recommended adjusting the sample dilution factor to be within this range.

- When a sample gives an OD below 0.6, it is recommended decreasing the dilution factor (i.e. 1/1000, 1/500 or 1/100).

- When a sample gives an OD beyond 1.8, it is recommended increasing the dilution factor (i.e. 1/5000, 1/10000 or 1/25000).

The standard curve



Result assessment

The measurement of total IgE requires testing the samples at least in duplicates. For high precision measures running a sample in triplicates is recommended. Repeated test of a sample in the same testing run are expected to provide results within +/-20% variability.

Once the concentration of total IgE is assessed for each sample an average is performed to determine the final total IgE concentration.

Total IgE test protocol

Step	Component or reagent	Volume (µl)	Comments	Incubation time	Incubation temp.
1	Sample 1/3000 dilution	100		o/n	4°C
2	Washing buffer	300	1 cycle		RT
3	Fc-epsilon receptor	100	Dilution: 1/250	30 minutes	RT
4	Washing buffer	300	1 cycle		RT
5	Streptavidin-Alkaline Phosphatase	100	Dilution: 1/250	30 minutes	RT
6	Washing buffer	300	4 cycles		RT
7	Chromogen (pNPP)	100	Ready to use	45 minutes	RT
8	L-Cys	50	Ready to use	Immediate	RT
9	OD reading at 405 nm				