HyCult biotechnology b.v.

HBT ELISA TEST KIT FOR IgY

The Hbt chicken IgY kit has been developed for the quantitative measurement of chicken IgY. The assay can be used to quantify chicken IgY during various purification steps of IgY.
The yolk of eggs laid by immunized chickens has been recognized as an excellent source of polyclonal antibodies (pAb). Specific antibodies produced in chickens offer several important advantages over producing antibodies in other mammals. Because a single egg contains as much antibody as an average bleed from a rabbit, this simple, non-invasive approach presents an appealing alternative to conventional pAb production methods. Purification of chicken egg yolk immunoglobulin Y (IgY), the 150 kDa IgG homolog, does not require animal bleeding. In addition, the eggs from immunized chickens provide a continual, daily source of pAb, and this convenient approach offers greater compatibility with animal protection regulations. Due to the phylogenetic distance between birds and mammals, there is greater potential of producing a higher percentage of specific antibody against mammalian antigens when using chickens. Highly conserved mammalian proteins sometimes fail to illicit a humoral response in animals, such as rabbits, that are traditionally used for generating pAb. Non-specific binding and need for cross-species immunoabsorptions is eliminated since chicken IgY does not cross-react with mammalian IgG and does not bind bacterial or mammalian Fc receptors. There are well defined structural differences of IgY-type immunoglobulins and the IgG of mammals. That includes the molar mass of the heavy chains of the immunoglobulins. The IgY-type immunoglobulins are much less flexible than IgG. Also, the structures of the Fc part of the immunoglobulin isotypes IgY and IgG are different.

PRINCIPLE OF THE TEST

The Hbt chicken ELISA is a solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. Samples and standards are incubated in microtiter wells coated with antibodies recognizing chicken IgY. During this incubation chicken IgY is captured by the solid bound antibody. Unbound material present in the sample is removed by washing. Biotinylated second antibody (tracer) to chicken IgY is added to the wells. If chicken IgY was present in the sample, the tracer antibodies will bind to the captured IgY. Excess tracer is removed by washing. Streptavidin-peroxidase conjugate is applied to the wells, this conjugate reacts specifically with the biotinylated tracer antibody bound onto the detected IgY. Excess streptavidin-peroxidase conjugate is removed by washing and substrate, tetramethylbenzidine (TMB) is added to the wells. Colour develops proportionally to the amount of chicken IgY present in the sample. The enzyme reaction is stopped by the addition of citric acid and the absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance versus the corresponding concentrations of the IgY standards.
The chicken IgY concentration of samples with unknown concentrations, which are run concurrently with the standards, can be determined from the standard curve.

SPECIAL FEATURES OF THE KIT

- Ready-to-use (ie. pre-coated microwells).
- High specificity for IgY due to the use of two monoclonal antibodies directed against different epitopes on the IgY molecule.
- The minimum concentration which can be measured is 10 ng/ml IgY.
- Large measurable concentration range. Standard curve from 8-2,000 ng/ml.
- Efficient format. 2 plates with twelve disposable 8-well strips allow free choice of batch size for the assay.
- Standardization. The calibration standards have been standardized by Hbt chicken IgY.
- High reproducibility.
- Simple, rapid procedure. Four pipetting steps are required to complete the assay. Working time 3½ hours.

AVAILABILITY: The Hbt chicken IgY test is available in kit for 2x96 determinations.

PRODUCT NUMBER: HK502 Hbt Chicken IgY test kit
For research purposes only.
Caution: Not for use in humans.