GENTAUR

DATA SHEET

Antisera for immunochemical detection of animal and plant proteins for food.

The following antisera are available for detection of protein additives in foods:

R 45261 - Antiserum to hazelnut corylin

These specific antisera are liquid animal sera, and as a rule are produced by immunizing rabbits with suitable commercial products or self-produced protein fractions. In order to obtain as wide an antibody population as possible against the various antigen determinants of the particular protein present in foods, the immunization material consists of a mixture of 'intact' commercial products of protein fractions and the same antigens which have been denatured by heating.
Nonspecific antibodies formed during immunization are removed by means of immunological absorption processes. Here, particular care is taken to avoid cross-reactions with closely related antigens, for example bovine/goat IgG oder bovine/Iporcine serum albumin.
The antisera for immunochemical detection of proteins have an antibody content that remains largely unchanged from batch to batch (Lot No.). The range of variation from batch to batch is about ± 30%.
Unstable serum proteins, particularly lipoproteins, are removed from the antisera by means of a special stabilisation process. Contamination with micro-organisms is largely precluded by sterile filtration and the addition of preservatives.

Preservatives:

1. sodium azide (< 1 g/l) (When using in-vitro diagnostics containing sodium azide the following should be considered: Avoid swallowing and contact with the skin or mucous membranes!)
2. sodium p-(ethylmercurythio)-benzenesulfate (max. 0.1 g/l)

Stability and Storage

The antisera will keep up to the date of expiry listed in the lable if stored at 2 - 8°C. Once a bottle of antiserum has been opened, it must be closed again tightly and returned to the storage of 2 - 8°C. It is preferable, but not absolutely necessary, to store them at –20°C after opening. This applies in particular in the case of time intervals of few weeks until reuse.

Method

Principle

The detection of proteins with the antisera is based on the specific reaction between antigen and antibody, which generally leads to an insoluble immunoprecipitate. The reaction is carried out in the liquid phase, e.g. in agarose gel, about 1.0 – 1.5 %.

Specificity

The high selectivity of the antigen/antibody reaction permits a clear immunochemical differentiation of the antigens under examination. If customary precipitation techniques are used, the conditions in the reaction medium must be approximately physiological, i. e.: pH approx. 6.6 to 9.0, salt concentration approx. 0.05 to 0.25 mol/l, temperature approx. +10 to +30°C. All substances which are able to cleave non-covalent bonds (e. g. the hydrogen bond) can disturb or prevent the reaction in the examination material, e. g. pH values of less then 4.0 or more than 9.0, higher salt concentrations (> 1 mol/l), and the presence of the detergents or other surface-active substances. Problems may also occur as a result of precipitates not due to antigen-antibody reactions, for example as a result of lectins.

Pretreatment

The antisera are ready for use in undiluted form. The antisera precipitate optimally at an antigen concentration of approx. 0.02 to 0.3 g/100 ml. It is highly protein-dependent, for example owing to differences in the molecular weight or in the carbohydrate content. If the antigen concentration is higher, it may prove advisable to dilute the respective specimen. A step towards higher extract concentrations is also possible.

Procedure

The following precipitation techniques can be used with the antisera.

1. Agar gel double diffusion according to Ouchterlony
   To a first approximation, the physical laws of free diffusion are applicable here. The antigen solution and antiserum are pipetted into opposite application points. The first volume is about 20 – 50 µl. Antigen proteins (control or sample) and antibody proteins from the antiserum diffuse towards one another in an agarose gel (plate size: slides). After completion of the diffusion – as a rule overnight – the immunoprecipitate remains locally fixed. The precipitates can be stained for better visualisation and documentation (1, 2).

2. Immunoelectrophoresis in the modification according to Scheidegger
   This method represents a combination of electrophoresis and immunodiffusion (1). In a first step, the antigens which are present in the punched holes on the agarose gel plate (size: microscope slide, fill volume: about 3 – 5 µl) are electrophoretically separated by applying an electric charge. A groove is then punched between the application points and is filled with antibody solution (antiserum). The plate is the allowed to stand so that the diffusion can take place – as a rule overnight. The antigen and antibody form precipitate arcs. This method can be used to compare complicated antigen mixtures (1, 3). Changes in the isoelectric point due to denaturing can also be analysed.

3. Radial immunodiffusion according to Mancini (4)
   The simple radial immunodiffusion is used for the quantitative determination of antigens and for determining the titre of antisera (3, 5). A dilution series of the antigen is introduced into small troughs in an agarose gel which contains the specific antiserum. The plates are allowed to stand for the last 24 hours. While the antigen diffuses radially out of the application points, precipitate rings are formed and appear to migrate further outwards until they finally come to a stop at the equivalence point at the end of the diffusion. If the area or the square of the diameter of the precipitate areaola is plotted against the antigen concentration, a straight line is obtained as a reference line which can be used to determine the antigen concentration in unknown solutions (2).

4. Immunoassay according to Laurell (2, 3)
   The immunoassay according to Laurell is a quantitative immunochemical method for the determination of antigens. The antigen solution is introduced into punched application points in an agarose gel which contains the specific antiserum to the required antigen. Under direct current conditions, antigens with a low isoelectric point ('acidic antigens') migrate to the anode while the antibodies generally remain stationary in the gel. This gives a long, rocket-like immuniprecipitate, the area of the precipitate being directly proportional to the antigen concentration. As a simplification, the height of the rock peak can be used as a measure of the antigen concetration.

5. Two-dimensional immunoelectrophoresis according to Clarke-Freeman (1, 2)
   This is a two-phase electrophoresis. In the first phase the electrophoretic separation of the specimen is carried out in a gel medium free of antiserum (analogous to immunoelectrophoresis). For the second phase, an antiserum-containing agarose gel is used. The proteins separated in the first phase are drawn into the agarose gel in the direction of the anode under electrophoretic direct current conditions. Precipitate arcs are formed. The area under these arcs is a measure of the concentration of the particular protein in the specimen.

Problems/Controls

The precipitate should be washed several times in isotonic saline solution, and then stained with Amido Black (Naphtol blue-black Ad.-No. 32691) or Coomassie Brilliant Blue R, in order to preclude non-specific precipitates which are not based on the reaction between antigen and antibody, and also in order to increase the sensitivity of the procedure. In tests for foreign protein additives, omitting to stain then immunoprecipitates, can lead to erroneous interpretations, since some proteins tend to precipitate non-specifically in a gel medium, especially if they had been denatured by heating. As a rule, washing several times with isotonic saline solutions will completely remove such non-specific precipitates.

It is possible to distinguish between specific immunoprecipitates and non-specific protein-protein or protein-carbohydrate precipitates by means of a control procedure in which the specific antiserum is replaced by an animal serum free from antibodies, for example rabbit serum. If precipitates result, and remain after washing and staining, they cannot be immunoprecipitates. In such a case, the result with the corresponding antiserum is attributable to factors other than those assumed in accordance with the labelling. In some cases it is advisable to allow the antiserum to pre-diffuse for about 3 to 4 hours by the Ouchterlony method. The low molecular weight antigen-containing solutions (for example in the case of casein or of stronger hydrolytically degraded protein fragments) are then introduced.

Analytical Significance of the Reagents

The reagents for immunochemical analysis of animal and plant proteins make it possible to detect such antigens in a practically unlimited range of foods, where they are found partly as prohibited additives. Here it must be kept in mind that the characteristics of the antigens in the material being examined can be altered considerably by heat denaturing or other measures. Such treatment can modify the previously intact immunochemicals structures of the protein which define the antigen determinants/epitopes. If hydrolysis is carried out at a high temperature, fragmentation cannot be precluded. This in a turn may lead to a loss of the antigen determinants originally present and, on the other hand, to the appearance of new antigen determinants. In addition, the antigens used as foreign proteins can be markedly altered immunologically as a result of variations in such factors as yearly harvests, the extent to which the product has been allowed to ripen, and various manufacturing processes (including chemical processing), which may also be frequently modified.

Although these factors are taken into account by the use of intact antigens and artificially denatured materials of the same antigen during the immunization process, extreme caution should always be exercised in interpreting immunoanalytical findings. The result obtained should be verified by running concomitant controls (identity or partial identity). Owing to the above-mentioned possibilities of denaturing and alternation of the proteins in the specimens, the manufacturer assumes no liability for erroneous evaluations of the test.

References

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