Anti-Glutamic Acid Decarboxylase (GAD) ELISA (IgG)

**Indication:** Test system for the in vitro determination of antibodies against glutamic acid decarboxylase (GAD) in human serum or EDTA plasma for the diagnosis of the following disease: diabetes mellitus type I.

**Clinical significance:** Diabetes mellitus type I (insulin-dependent diabetes mellitus, IDDM) is an autoimmune disease which is accompanied by destruction of the pancreas islets through autoimmune T cells and the formation of autoantibodies. It occurs predominantly in young people and represents 10% of all diabetes cases. The determination of autoantibodies against pancreas islet cell antigens is used to confirm the diagnosis of type I diabetes and to identify preclinical autoimmune reactions in persons at risk. In most cases one or more diabetes-mellitus-associated autoantibodies can be detected at the time of clinical manifestation.

**Autoantibodies against pancreas islet cells (ICA)** can be detected in 80% of patients with newly onset diabetes using indirect immunofluorescence. Two target antigens for ICA have been so far identified — the enzymes glutamic acid decarboxylase (GAD) and tyrosine phosphatase (IA2). GAD catalyzes the synthesis of the neurotransmitter gamma aminobutyric acid and consists of two isoforms, GAD65 and GAD67. GAD65 has been shown to be the major target for autoantibodies against GAD in type I diabetes.

Autoantibodies against glutamic acid decarboxylase (GADA) occur in type I diabetes mellitus at a prevalence of 60–85%, but they were first observed in Stiff-Man syndrome. Autoantibodies against tyrosine phosphatase (IA2A) are not associated with Stiff-Man syndrome. Their prevalence in type I diabetes is 48–80%, whereas they are more frequently detected the younger patients are. The prevalence of **autoantibodies against insulin (IAA)** is also age dependent. In patients under 5 years of age the prevalence amounts to over 90%, while by the 12th year it decreases to 40%. IAA are therefore of great relevance in paediatrics.

Autoantibodies associated with type I diabetes mellitus can be detected years before clinical manifestations appear. Their determination enables early identification of persons at increased risk. Through suitable intervention, for example maintaining glucose concentrations at a low level or implementing immunosuppressive measures, the development of the disease can be avoided in some cases. To be able to evaluate the possible risk of diabetes in an individual case, parallel testing for several of the relevant antibodies (GADA, IA2A, IAA, ICA) is recommended. Thus the diagnostic sensitivity and specificity for the diagnosis of a type 1 diabetes can be significantly increased.

**Correlation of the EUROIMMUN Anti-GAD ELISA with Anti-IgG-GAD RIA:** Sera from 39 patients with type I diabetes mellitus, 74 patients with suspected type I diabetes mellitus, 62 patients with type II diabetes mellitus, 119 patients with other autoimmune diseases and 300 healthy blood donors were investigated for GADA using the EUROIMMUN ELISA and RIA. There was a very high correlation between the EUROIMMUN Anti-GAD ELISA and RIA. Linear regression analysis of ELISA and RIA results in the 39 selected type I diabetes mellitus sera showed the following correlation characteristics: y = 5.2x−258; p < 0.001; r = 0.9.

**2005 Diabetes Autoantibody Standardization Program (DASP):** The 150 sera from the DASP (50 sera from patients with newly diagnosed type I diabetes, and 100 sera from healthy blood donors) were assayed and results showed a sensitivity of 92% and a specificity of 98% for the ELISA, and a sensitivity of 84% and a specificity of 95% for an Anti-IgG-GAD RIA, which uses the same recombinant antigen as the ELISA.
**Test Characteristics**

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**Principle and procedure:** The Anti-GAD ELISA is a quantitative in vitro assay for GADA. Patient serum is incubated with GAD coated onto a microplate. If sample is positive, specific antibodies bind to the GAD. Bound antibodies are able to act divalent and form a bridge between GAD on microplate well and biotin-labeled GAD, which is added in a second incubation step. To detect the bound biotin, a third incubation is carried out using enzyme-labeled avidin, which is capable of promoting a color reaction. The intensity of the color formed is proportional to the antibody concentration.

**Reproducibility:** Coefficients of variation (CV) were determined using data from 2 sera with values at different points on the calibration curve. The intra-assay CVs are based on 25 determinations and the inter-assay CVs on 20 determinations.

**Reference ranges:** Levels of GADA were analyzed in sera from 300 healthy blood donors using the EUROIMMUN ELISA. 99% of these sera gave concentrations of less than 10 IU/ml. In addition, sera from 39 patients with a definite diagnosis of type 1 diabetes mellitus (patient group 1) and 74 sera from patients with suspected type 1 diabetes mellitus (patient group 2) were analyzed. In the ELISA 90% of group 1 and 50% of group 2 gave responses greater of than 10 IU/ml.

**Technical data:**

**Antigen**
The microplate wells were coated with human recombinant glutamic acid decarboxylase (isoform GAD65). The same antigen is used in the ELISA in biotinylated form. The corresponding cDNA was expressed in yeast.

**Sample dilution**
Serum, EDTA plasma; 25 µl undiluted.

**Calibration**
Quantitative, in international units per ml (IU/ml).

**Reagents**
Ready to use, with the exception of the wash buffer (10x), GAD (lyophilized) and enzyme conjugate (20x).

**Test procedure**
1 hour / 1 hour / 20 min / 20 min. (Sample / GAD / conjugate / substrate incubation) *1st, 2nd and 3rd incubation on a microplate shaker set at 500 rpm. Room temperature.

**Measurement**
450 nm (GADA conc. <35 IU/ml) and 405 nm (GADA conc. >35 IU/ml). Reference wavelength 620 – 650 nm.

**Kit format**
96 individual break-off wells.
Kit includes all necessary reagents.

**Order no.**
EA 1022-9601 G

**Related products**
EA 1023-9601 G (Anti-IA2 ELISA)
EA 1022-9601-1 G (Anti-GAD/IA2 Pool ELISA)