# DRG® Islet Cell Autoantibodies (ICA) ELISA (EIA-1594)

USA: RUO

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***This kit is intended for Research Use Only.***

***Not for use in diagnostic procedures.***

***Please use only the valid version of the package insert provided with the kit.***

## 1 INTRODUCTION AND INTENDED USE

DRG´s Islet Cell Autoantibodies (ICA) ELISA is a qualitative ELISA test for measurement of circulating IgG antibodies against pancreatic islet cell antigens.

## 2 PRINCIPLE OF THE TEST

A purified mixture of pancreatic antigens is immobilized onto microwells. During an incubation period, antibodies in the serum sample are allowed to react at room temperature with antigen molecules on the microwells. After washing off excess/unbound serum materials, an enzyme (alkaline phosphatase) labeled goat antibody, specific to human IgG, is added to the antigen-antibody complex. After another thorough washing, a substrate (PNPP) is added and the color generated is measured spectrophotometrically. The intensity of the color is directly proportional to the concentration of ICA in the sample. An ICA-high-analyte control serves as an internal quality control and ensures valid results.

## 3 WARNING AND PRECAUTIONS

### *1.* Potential Biohazardous Material

The matrix of the Calibrators and Controls is human serum. The human serum used has been found non-reactive to HbsAg, anti-HIV 1/2 and anti-HCV when tested with FDA licensed reagents. Because there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled as if potentially infectious.

### *2.* Sodium Azide

Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.

### *3.* Stop Solution

Stop Solution consists of 1N NaOH. This is a strong base and should be handled with caution. It can cause burns and should be handled with gloves. Wear eye protection and appropriate protective clothing. Avoid inhalation. Dilute a spill with water before absorbing the spill with paper towels.

### Precautions

1. Do not freeze test reagents, store all kit components at 2 °C - 8 °C at all times.
2. High-analyte and Low-analyte Controls must be run each time the test is performed.
3. Use only clear serum as test specimens. The test sample should not have gross turbidity, hemolysis, or microbial contamination.
4. All samples should be analyzed in duplicate.

5. Do not mix reagents from different lots.
6. Do not use expired reagents.
7. Do not allow reagents to stand at room temperature for extended periods of time.
8. Do not expose substrate solution to light.
9. Careful pipetting technique is necessary for reproducible and accurate results.

## 4 REAGENTS AND MATERIALS

**Materials Supplied:**

| | | | |
|---|---|---|---|
| 1. | **PLA ICA** | **Microwell Strips (with the holder)** | **12 strips** |
| 2. | **CONJ ENZ 6X** | IgG Enzyme Conjugate (6X conc.) | 2 x 1.0 mL |
| 3. | **DIL SPE 5X** | Sample Diluent (5X concentrate) | 1 x 25.0 mL |
| 4. | **CONJ ENZ DIL** | Conjugate Diluent | 1 x 10.0 mL |
| 5. | **CTRL REF ICA** | Reference Control | 1 x 1.5 mL |
| 6. | **CTRL + ICA** | High-analyte Control (human serum) | 1 x 1.5 mL |
| 7. | **CTRL – ICA** | Low-analyte Control (human serum) | 1 x 1.5 mL |
| 8. | **SUBS PNPP** | Substrate Solution (PNPP) | 1 x 15.0 mL |
| 9. | **BUF WASH 25X** | Washing Buffer (25X concentrate) | 1 x 20.0 mL |
| 10. | **SOLN STP** | Stop Solution (1N NaOH) | 1 x 6.0 mL |

## 5 ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Distilled or deionized water.
2. Absorbent paper towels to blot dry the strips after washing and parafilm/plastic wraps to cover strips during incubations.
3. Suitable sized glass tubes for serum dilution.
4. Micropipette with disposable tips to deliver 10 µL, 50 µL and 100 µL.
5. A microtiter plate washer or a squeeze bottle for washing.
6. 5 mL pipettes for conjugate diluent delivery.
7. A 500 mL graduate cylinder.
8. Microtiter plate reader with 405 nm absorbance capability.
9. Plastic label tape, to tape unused wells before assay.

## 6 SPECIMEN COLLECTION

Collect 5-10 mL of blood by venipuncture into a clot (red top) tube. Serum separators may be used. Separate serum by centrifugation.

Serum samples may be stored at 2 °C - 8 °C.

Excessive hemolysis and the presence of large clots or microbial growth in the test specimen may interfere with the performance of the test.

Freeze the serum sample at -20 °C if it cannot be analyzed within 24 hours.

## 7 REAGENT PREPARATION AND STORAGE

### *1.* IgG Enzyme Conjugate Reconstitution:

Accurately transfer 5 mL of the Conjugate Diluent into one bottle containing the IgG Enzyme Conjugate (concentrate). Close the bottle and mix thoroughly by inversions.
Store the diluted conjugate at 2 °C - 8 °C when not in use. Record the date of reconstitution on the label.
**This diluted reagent expires 30 days after reconstitution.**
Two bottles containing the conjugate concentrate are provided. Each bottle contains enough conjugate for 6 strips. Reconstitute as needed.

### *2.* Sample Diluent Buffer:

If precipitate is present in the sample diluent buffer concentrate due to storage at lower temperature such as 2 °C - 8 °C, dissolve by placing the vial in a 37 °C water bath for 30 minutes.

Transfer the entire contents (25 mL) into 100 mL of distilled/deionized water in a suitable container.
Mix thoroughly; label the container as Sample Diluent, and store at 2 °C - 8 °C.
The diluted reagent is stable until the expiration shown on the vial.

Please note that the precipitate seen in the concentrate has no effect on the performance of the test and will not be present in the 1X working solution.

### *3.* Wash Solution:

Transfer the entire contents into 480 mL of distilled/deionized water in a 500 mL container. Mix thoroughly; label the container as Wash Solution, and store at 2 °C - 8 °C.
The diluted reagent is stable until the expiration shown on the vial.

### *4.* Serum Sample Preparation:

Accurately pipet 10 µL (0.010 mL) of serum sample into 1.0 mL of the Working Sample Diluent into an already labeled glass tube. Mix thoroughly.

## 8 ASSAY PROCEDURE

The test kit contains 12 microwell strips coated with purified islet cell antigens. The number of microwell strips used in each assay depends upon the number of serum samples to be tested. If 12 microwell strips are used, a total of 45 sample sera can be tested in duplicate with this kit.

**IMPORTANT NOTE:** Bring all the reagents, including serum samples, to room temperature (25 °C) before starting the assay. Incubation temperatures varying by greater than ± 1 °C can definitely affect results.

1. Assemble the number of microwell strips needed for the test in the holder provided. The microwell strip must be snapped in place firmly or it may fall out and break.
2. Familiarize yourself with the indexing system of wells, e.g. well #A1, B1, C1, D1, etc.
3. Dispense **100 µL of Low-analyte Control** into microwells C1 and D1.
4. Dispense **100 µL of High-analyte Control** into microwells E1 and F1.
5. Dispense **100 µL of Reference Control** into microwells G1 and H1
6. Add **100 µL of diluted sample serum** (see #4, Section 7, Reagent Preparation) to microwells starting from A2 and B2. For more specimen samples, use additional strips and add other diluted specimen samples to microwells in duplicate.
   There should be 100 µL of solution in each microwell to be assayed except A1 and B1 which are empty at this point and will be used later.
7. Any strips not used should be properly stored with desiccant in the ziplock bag provided for the next run. Any wells not used on the strip should be properly covered and saved for the next run.
8. Cover the plate with a parafilm/plastic wrap (to prevent contamination) and leave for **1 hour at room temperature (25 °C ± 1 °C).**
9. After incubation, discard the solution into sink by quick decantation and blot the plate dry by tapping gently onto a paper towel.
   If an automatic plate washer is being used, **wash** each well **3 times** with **300 µL (0.3 mL) of the Wash Solution**.
   If a squeeze bottle is used, fill the wells with the Wash Solution carefully and decant the buffer from the microwells. Repeat the procedure two more times and blot the plate dry with a paper towel.
10. Add **100 µL of IgG Enzyme Conjugate** reagent (see #1, Section 7, Reagent Preparation) to all microwells except wells A1 and B1.
11. Cover the plate with a parafilm/plastic wrap and let it stand at **room temperature (25 °C ± 1 °C) for one hour**.
12. After incubation, **repeat** the **washing step** (step #9) and blot the microwells dry.
13. Add **0.1 mL (100 µL) of Substrate Solution** to all microwells including wells A1 and B1. Be sure to dispense the substrate reagent at a rapid steady pace without any interruption.
14. Cover the plate and leave it in the dark for **30 minutes at room temperature** (25 °C ± 1 °C).
15. After 30 minutes promptly add **50 µL of the Stop Solution** into each well at a rapid steady pace without any interruption.
16. Set up microplate reader to **read the absorbance at 405 nm** according to manufacturing instructions, and blank the plate reader with well A1 or B1.
17. Calculate the data according to Section 9.

## 9 CALCULATION OF DATA

Record the spectrophotometric readings [optical density (OD) in absorbance units] as shown in the example (ICA Sample Data). The actual OD reading from your ICA ELISA may be different. This is only an example.

1. Calculate the average O.D. reading of the Reference, Low-analyte and High-analyte Controls and Specimen samples done in duplicate.

   The average reading (mean) of the Reference Control is $R_m$,
   of the Low-analyte Control is $N_m$,
   of the High-analyte Control is $P_m$, and
   of sample data is $S_m$.

2. Divide the average O.D. of Samples and Controls by the $R_m$ value. This gives a Ratio Value for each sample.

**ICA ELISA - SAMPLE DATA**

Section A: Control Results

| Data | | | | |
|---|---|---|---|---|
| **Controls** | **O.D.** | **Ave. O.D.** | **Ratio value** | **Result** |
| Reference Ctrl | 1.072<br>1.092 | $R_m$ = 1.082 | 1.00 | |
| Low-analyte Ctrl | 0.290<br>0.303 | $N_m$ = 0.297 | 0.27 | Low-analyte |
| High-analyte Ctrl | 1.413<br>1.406 | $P_m$ = 1.409 | 1.30 | High-analyte |

Note: For a valid test, the ratio value for $N_m$ should be < 0.95 and $P_m$ > 1.05.
Repeat the test if results are not valid.

Section B: Specimen Sample Results

| Data | | | | |
|---|---|---|---|---|
| **Sample** | **O.D.** | **Ave. O.D.** | **Ratio value** | **Result** |
| Reference Ctrl | 1.072<br>1.092 | $R_m$ = 1.082 | 1.00 | |
| 1 | 1.444<br>1.472 | $S1_m$ = 1.458 | 1.35 | High-analyte |
| 2 | 0.549<br>0.534 | $S2_m$ = 0.541 | 0.50 | Low-analyte |
| 3 | 1.036<br>1.051 | $S3_m$ = 1.043 | 0.96 | Intermediate |

## 10 QUALITY CONTROL

Low-analyte and High-analyte Controls must be run along with unknown samples each time in order for results to be valid.
The Low-analyte Control should show a ratio value < 0.95 and the High-analyte Control should show a value > 1.05.

## 11 PERFORMANCE CHARACTERISTICS

The specificity of antigen coated ICA microwell strips was established by Western blot analysis using confirmed high-analyte samples for IgG to Islet Cell Antigens.

Samples with thyroid autoantibodies and rheumatoid factors read low-analyte on Islet Cell Autoantibodies (ICA) ELISA.

## 12 LIMITATIONS AND SOURCES OF ERROR

1. Although a higher ICA titer will produce a higher O.D. reading, the test is designed for qualitative determination of ICA only.
2. Poor test reproducibility may result from:
   a. Inconsistent delivery of reagents;
   b. Improper storage of reagents;
   c. Improper reconstitution of reagents;
   d. Incomplete washing of microwells;
   e. Substrate reagent old or exposed to light;
   f. Unstable/defective spectrophotometer;
   g. Error in following the assay procedure.

## 13 LITERATURE

1. Orci, L., J. Vassali, and A. Parrelet (1988). The insulin factory. Sci. Amer., 261:85-94.

2. Eisenbarth, G.S. (1985). Type I diabetes: A chronic autoimmune disease. New Engl. J. Med., 314:1360-1368.
3. Eisenbarth, G.S., J. Connelly, and J.S. Soeldner (1987). The natural history of Type I diabetes. Diabet./Metab. Rev., 3:873-891.
4. Etzioni, A. (1987). Immunological concepts in insulin-dependent (Type I) Diabetes Mellitus Immunol Res., 6:224-251.
5. Rossini, A.A., J.P. Mordes, and E.S. Handler (1989). A "Tumbler" hypotheses: The autoimmunity of insulin-dependent diabetes mellitus. Diabet. Spect., 2:195-201.
6. Soeldner, J.S. (1985). The prodermal phase of insulin-dependent diabetes. In: Current Medical Literature - Diabetes, 2:66-70.
7. Colman, P.G., R.C. Nayak, J. Connelly, A. Rabizadeh, J.S. Soeldner, and G.S. Eisenbarth (1986). Islet cell antibodies: Clinical utility and target antigens. In: The Immunology of Diabetes Mellitus (Jaworski, M.A. ed.). Elsevier, NY, 345-350.
8. Palmer, J.P. (1987). Insulin autoantibodies: Their role in pathogenesis of IDDM. Diabet./Metab. Rev., 3:1005-1015.
9. Srikanta, S., A.T. Ricker, D.K. McCulloch, J.S. Soeldner, G.S. Eisenbarth, and J.P. Palmer (1986). Autoimmunity to insulin, beta cell dysfunction, and development of insulin-dependent diabetes mellitus. Diabetes, 35:139-142.
10. Dean, B.M., F. Becker, J.M. McNally, A.C. Tarn, G. Schwart, E.A.M. Gale, and Bottazo, G.F. (1986). Insulin autoantibodies in the pre-diabetic period: correlation with islet cell antibodies and development of diabetes. Diabetologia, 29:339-342.
11. Irvine, W.J., R.S. Gray, and J.M. Steel (1980). Islet cell antibody as a marker for early stage Type I diabetes. In: The immunology of diabetes (Irvine, W.J., ed.), Teviot, Edinburgh, pp. 117-154.
12. Kamalesh, M., A. Karasik, and S. Srikanta (1986). Islet cell antibodies as predictors of insulin-dependent diabetes mellitus. Pract. Cardiol., 12:79-91.
13. Irvine, W.J., C.J. McCallum, R.S. Gray, C.J. Campbell, J.J.P. Duncan, J.W. Faraquar, H. Vaughan, and P.J. Morris (1977). Pancreatic islet cell antibodies in diabetes mellitus correlated with duration and type of diabetes, coexistant autoimmune disease and HLA type. Diabetes, 26:138-147.
14. Landrum, R., G. Walker, A.G. Cudworth, C. Theophanides, D.A. Pyke, A.J. Bloom, and D.R. Gambler (1976). Islet cell antibodies in diabetes mellitus. Lancet, 2:1273-1276.
15. Riley, W. and N. MacLaren (1984). Islet cell antibodies are seldom transient. Lancet, 1:1351-1352.
16. Soeldner, J.S., M. Tuttleman, S. Srikanta, O.P. Ganda, and G.S. Eisenbarth (1985). Insulin-dependent diabetes and initiation of autoimmunity: Islet cell autoantibodies, insulin autoantibodies and beta cell function. New Engl. J. Med., 313:893-900.
17. Battazo, G.F., R. Pujol-Barrell, and E. Gale. In: The Diabetes Annual (K.G.M.M. Alberti and L.P. Krall, eds.), Elsevier, NY, Oxford, 1:16-52.
18. Srikanta, S., O.P. Ganda, R.A. Jackson, R.E. Gleason, M.E. Kaldany, M.R. Garovoy, E.L. Milford, C.B. Carpenter, J.S. Soeldner, and G.S. Eisenbarth (1983). Type I diabetes mellitus in monozygotic twins: chronic progressive beta cell dysfunction. Annals Int. Med., 99:320-326.
19. Srikanta, S., O.P. Ganda, A. Rabizadeh, J.S. Soeldner, and G.S. Eisenbarth (1985). First-degree relatives of patients with Type I diabetes mellitus: Islet cell antibodies and abnormal insulin secretion. New Engl. J. Med., 313:461-464.

20. Riley, W.J., N.K. MacLaren, and J.H. Silverstein (1988). The predictability of insulin-dependent diabetes mellitus. Adv. Pediatr., 35:167-187.
21. Boitard, C., E. Bonifacio, G.F. Bottazo, H. Gleichman, and J. Molenaar (1988). Immunology and diabetes workshop: Report on the third international (stage 3) workshop on standardization of cytoplasmic islet cell antibodies. Diabetologia, 31:451-452.
22. Bottazo, G.F., A. Florin-Christensen, and D. Doniach (1974). Islet cell antibodies in diabetes mellitus with polyendocrine deficiencies. Lancet, II:1279-1283.
23. Srikanta, S., A. Rabizadeh, M.A.K. Omar, and G.S. Eisenbarth (1980). Assay for islet cell antibodies: Protein A-monoclonal antibody method. Diabetes, 34:300-305.

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