# FAQ: Frequently Asked Questions

## Device: ICA ELISA (REF 7010)

### Sample Type

***What type of samples can I collect and test?***
The determination of ICA should be performed on human serum. Collect blood by venipuncture in a clot (red top) tube. Serum separators may be used but do not use anticoagulants or preservatives. The serum should be promptly separated by centrifugation. Serum can be stored at 2-8°C for up to 1- days or frozen at ≤ -20°C. Freeze the serum samples if they cannot be analyzed within 24 hours of collection.

***What should I do if the test sample is hemolyzed or lipemic?***
Grossly hemolyzed, lipemic, and icteric samples as well as samples containing particulate matter or showing obvious microbial contamination should not be tested.

***How long can samples be stored before testing?***
Serum samples may be stored up to 10 days at 2-8°C. Serum samples frozen at -20°C are stable for up to 4 months.

***What is the minimum volume of sample required for testing?***
For duplicate testing, 20µL of sample is required.

***Can I test non-Human samples?***
At this time, Biomerica has not validated its ICA ELISA kit with the use of non-Human plasma or serum.

### Reagents

***Can I interchange lot numbers?***
ELISA kit components are lot specific. Do not interchange lot numbers from previous kits.

***What are the storage conditions for the kit components?***
All reagents except the ICA –Enzyme Conjugate, Isletest sample diluent, and Wash Concentrate are ready-to-use. Store all reagents at 2-8 °C.

***Can I freeze the kit reagents?***
No, the kit reagents should be stored between 2°C and 8°C.

***Can I reuse microwell strips?***
No. Unused microwell strips must be placed back inside the resealed foil pouch. It is important to protect the strips from moisture.

***How quickly should I pipette calibrators, controls and samples into the wells?***
Bring all reagents to room temperature (25°C) before starting the assay.

***What is the control source in the ICA ELISA?***
The control source in the ICA ELISA is human serum. This kit does not have any recombinant reagents.

***What is the stability of the working wash solution?***
The diluted working wash solution is stable at 2-8 °C through the shelf life.

***What is the storage of the reconstituted conjugate?***
The reconstituted conjugate is stable up to 30 days at 2-8 °C. Store the remaining diluted conjugate at 2-8 °C. The reconstituted conjugate is stable up 30 days.

***Is precipitate present in the wash concentrate normal?***
Precipitate present in the Wash Concentrate is normal. This is due to storage at lower temperature such as 4°C. Dissolve by placing the vial in a 37° C water bath. If incubating the wash concentrate in a water bath does not dissolve the precipitate, the precipitate will dissolve once the wash concentrate is diluted to a 1X solution. To eliminate precipitate formation, the wash concentrate can be stored between 2-30°C according to the label.

***What should I do if a reagent has no label, lot or expiration date?***
Do not use any reagents that lack a label, lot number or expiration date. Please contact Biomerica.

***What should I do if I receive a reagent that is leaking or broken***?
Do not use any reagents that appears to be leaking or broken. Please contact Biomerica.

## Sample Preparation

***How do I prepare the sample for testing?***
Pipette 10µL of serum to 1.0 mL working sample diluent buffer in glass tube. Mix thoroughly before plating.

## ELISA Testing Procedure

***How long does it take to perform the ICA ELISA?***
Assay time is 2 hour 30 minutes.

***What is the maximum number of samples that can be tested on one kit?***
If Positive Control, Negative Control, and Reference Control are run in duplicate and two wells are left for blanking, a total number of **45** samples can be run on one ELISA kit.

***How long do I have to let the reagents come to room temperature before starting the assay?***
Bring all reagents to room temperature before use to ensure proper kit reactivity. You may remove reagents from foam inset to reduce the time need to come to room temperature. Typical time to come to room temperature is 1 hour.

**Can I use an automated microplate washer when running the ELISA?**
Yes, an automated plate washer may be used. If a microplate washer is unavailable, manual washing is also acceptable.

***Can I run my assay via automated methods?***
Biomerica has only validated its ICA ELISA kit via manual testing as outlined in the approved IFU. You would need to perform your own validation to ensure equivalent performance between the manual method and the chosen automated method.

**Can I use incubation times and/or temperatures that are different than what are listed in the protocol?**
Using incubation times and/or temperatures that differ from what is listed in the Package Insert may give erroneous results, and is therefore discouraged.

**Can I read the assay at 450 nm instead of 405 nm?**
No, this assay must be read at 405 nm, not at 450 nm.

***What precautions should I take when running this kit?***
The source of the Calibrators and Controls is human serum, in which the human serum used has been found non-reactive to HbsAg, anti-HIV ½, and anti-HCV when tested with FDA licensed reagents. Although 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.

All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Common precautions in handling should be exercised, as applied to any untested patient sample.

Stopping Solution consists of 1 N NaOH. This is a strong base and should be handled with caution. It can cause burns and should be handled with gloves and eye protection, with appropriate protective clothing. Any spill should be wiped immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.

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.

Substrate Solution consists of para-Nitrophenylphosphate (PNPP), a non-proteinaceous chromogenic substrate used in ELISA test. On occasion substrate may display a yellow color. This color will not interfere with test results.

## Interpretation of Results

***What concentration units are expressed in the test result?***
The Biomerica ICA ELISA expresses the ratio value results in U/mL.

## Curve Fit, QC, and Data Analysis

***What is considered an invalid Test?***
In assays in which one or more of the quality control sample values lay outside the acceptable limits, the results for the patient sample should be considered invalid. For the Biomerica ICA ELISA, the Negative Control should show a ratio value < 0.95 U/ml and the Positive Control should show a value > 1.05 U/ml.

***What is recommended for internal QC of each kit run?***
It is recommended that control serum or serum pools should be evaluated with each run of controls and patient samples. Results generated from the analysis of the control samples should be evaluated for acceptability using appropriate statistical methods.

***What should I do if the sample result is Indeterminate?***
If an indeterminate (borderline) value is obtained, the sample should be retested or saved to run in parallel with a fresh patient sample at a later date.

## Performance Characteristics

***Does the ICA ELISA have cross-reaction with Rat or other non-human species of ICA?***
Unfortunately, we don't have data to support cross-reactivity with rat or other non-human species. This assay has only been validated for use in human serum.

## Limitation of Procedure

***What are some of the limitations of the Biomerica ICA ELISA?***
Like any analyte used as a diagnostic adjunct, ICA results must be interpreted carefully with the overall clinical presentations and other supportive diagnostic tests.

## Supplemental Information

**Can I purchase reagents individually?**
The GAD assay is intended to be sold as a complete kit. Please contact Biomerica for reagent availability

# Troubleshooting Tips

| Observation | Probable Cause |
|---|---|
| **Low Absorbance** | Enzyme or substrate reagent degradation |
| | Improper buffer/diluent used |
| | Reagents were cold when added to wells |
| | Incorrect incubation time |
| | Wells dried out |
| | Excessive wash/aspiration pressure from automated plate-washer |
| | Plate reader set at incorrect wavelength |
| **High Background** | Incomplete washing/aspiration of wells |
| | Substrate contamination |
| **Poor Precision** | Inaccurate pipetting |
| | Inadequate mixing of reagents |
| | Samples contain precipitates |
| | Wells dried out |
| | Contaminated well bottom |
| **Poor Standard Curve** | Incomplete washing/aspiration of wells |
| | Inaccurate pipetting |

# ICA ELISA Kit – Procedural Flow Chart