



RUO in the USA

Revised 14 Nov. 2011 rm (Vers. 5.1)

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 PRINCIPLE OF THE TEST

Anti-GAD₆₅ RIA is a direct assay based on the principle of radioligand assays. Highly purified human recombinant GAD₆₅ is labeled with I¹²⁵-Iodine. This tracer is used in excess and bound by the GAD₆₅ autoantibodies of the sample.

Anti-GAD₆₅ RIA tracer meets the highest requirements with regard to purity, enzymologic identity, fast reaction kinetics, cross reactivity at zero level and stability. These are the main prerequisites for the specific binding of the tracer and its exclusive recognition by the GAD₆₅ autoantibodies of the sample.

By adding Protein A (staphyl. aureus) which binds to the Fc moiety of the autoantibodies, sandwich-type complexes are formed. This solid phase facilitates the simple separation of the bound fraction (B) by centrifugation. After removing the supernatant which contains the non-bound tracer by aspiration or decantation, the radioactivity of the remaining precipitate is measured.

The concentration of GAD₆₅ autoantibodies (anti-GAD₆₅) in the sample is reflected by the specifically bound tracer amount. The radioactive signal (cpm) of the bound fraction (B) is proportional to the autoantibody concentration.

No immune complex is formed if autoantibodies against GAD₆₅ are absent in the sample, as the tracer binds solely to GAD₆₅ autoantibodies, but not to Protein A.

A standard curve with a range of 0.1 - 120 (300) U/ml is established by measuring cpm respectively the binding B/T % of the calibrators **1 - 6 (7)**. The anti-GAD₆₅ concentration value of the donor's sample is directly read off against this curve.

2 DONOR SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. After clotting, the serum is separated by centrifugation. Plasma is also suitable for use in Anti-GAD₆₅ RIA. Lipaemic and hemolytic samples should not be employed.

The samples may be kept at 2 - 8 °C up to three days. Long-term storage requires - 20 °C.

Repeated freezing and thawing should be avoided.

For multiple use, initially aliquot samples and keep at - 20 °C.



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3 TEST COMPONENTS FOR 100 (50) DETERMINATIONS

| | | |
|-----------------------------------|---|---|
| D TRAC | Tracer (125-I-GAD ₆₅ human, recombinant) < 0.05 MBq per vial, t _{1/2} = 59 days gamma radiation 35 keV, x radiation 27 keV, 31 keV | 2 (1) vials, lyophilized, Reconstitution: 2.6 ml buffer J, each |
| J BUF D | Buffer (for reconstitution of components D and L and for washing) | 1 bottle, 120 ml, ready for use |
| L PRE | Protein A-Suspension | 2 (1) vials, lyophilized, Reconstitution.: 2.6 ml buffer J, each |
| 1-7 CAL | Anti-GAD₆₅-Calibrators (human serum) conc.: 0.1; 1.0; 3.0; 10; 30; 120 U/ml (300 U/ml optional) | 7 vials; 0.15 ml, each, ready for use |
| CI – CII CONTROL | Anti-GAD₆₅-Control sera (human sera) <i>Conc.:</i> cf. leaflet enclosed | 2 vials, 0.15 ml, each ready for use |

3.1 Size and storage

Anti-GAD₆₅ RIA has been designed for 100 and 50 determinations, respectively. This is sufficient for the analysis of 41 or 16 unknown samples as well as for calibrators and control sera, assayed in duplicates.

The expiry date of each component is reported on its respective label, that of the complete kit on the box label.

Upon receipt, all components of the Anti-GAD₆₅ RIA have to be kept at 2 - 8 °C, preferably in the original kit box.

3.2 Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

A Tracer:

Reconstitute with 2.6 ml **J** per vial.

Reconstituted tracer remains stable for 2 weeks, stored at 2 - 8 °C.

B Buffer:

is ready for use and serves for the reconstitution of the tracer and the Protein A-suspension as well as for washing.



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C Protein A-Suspension:

Reconstitute with 2.6 ml **J** per vial.

The reconstituted suspension remains stable for 2 weeks stored at 2 - 8 °C.

Note: Protein A suspension tends to precipitate in rest, thus agitate bottle end over end gently for 10 - 20 seconds immediately before use. This is not necessary for the short time of taking aliquots for the assay procedure.

1 - 7 Calibrators: Ready for use.

Normally, the calibrators 1 - 6 (0.1 - 120 U/ml) are used for the preparation of the standard curve. Expecting high values of antibody titers the optional calibrator 7 (300 U/ml) can be use for larger range of the standard curve.

CI - CII Control sera: Ready for use.

4 ASSAY PROCEDURE

Use conical tubes for Anti-GAD₆₅ RIA only.

1. Label test tubes appropriately.
2. Pipette into the corresponding tubes according to assay scheme
 - 20 µl calibrators,
 - 20 µl control sera,
 - 20 µl donor's samples, each.
3. Add 50 µl tracer (prepared from D and J), each, to all tubes, including those for total radioactivity T.
Tubes T are now separated until radioactivity is measured.
4. Incubate for 2 hours (at room temperature).
5. Add 50 µl Protein A-suspension (prepared from J and L), each.
(Agitate the suspension gently prior to use - please cf. section Test Components, preparation before use).
6. Incubate for 1 hour (at room temperature).
7. Add 1 ml buffer (J), each.
8. Centrifuge the tubes for 20 minutes at a minimum of 1500 x g.
9. Aspirate supernatant completely or decant. For removal of any remaining liquid, turn tubes upside down (5 - 10 minutes) and absorb any droplets by tapping on blotting paper.
10. Measure radioactivity of **all tubes including T**.
Recommended counting time: 1 minute

5 DATA PROCESSING

The standard curve is established by plotting the mean cpm-values of the calibrators 1 - 6 (7) on the ordinate, y-axis, (log. scale) versus their respective anti-GAD₆₅-concentrations on the abscissa, x-axis, (log. scale, as well).

The anti-GAD₆₅ concentrations of the controls and the unknown samples are **directly read off** in U/ml against the respective cpm values.



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The respective binding rates B related to the total radioactivity T may be used as well for setting up the standard curve (B/T %).

Anti-GAD₆₅ RIA may be used also with Computer Assisted Analysis using software able to plot log/log curves with spline smoothing, such as for sandwich-type assays (IRMA).

We recommend log/log processing for best results!

TYPICAL EXAMPLE(approx. 4 weeks before expiry)

Do not use for evaluation!

| Test tubes | cpm (a) | cpm (b) | Cpm (mean) | B/T % | U/ml |
|--------------------------------|---------|---------|------------|-------|------|
| Total radioactivity T | 29951 | 29878 | 29914 | 100 % | -- |
| Calibrator 1 | 1580 | 1483 | 1532 | 5.1 | 0.1 |
| Calibrator 2 | 2633 | 2692 | 2663 | 8.9 | 1 |
| Calibrator 3 | 4125 | 4144 | 4134 | 13.8 | 3 |
| Calibrator 4 | 8095 | 8989 | 8163 | 27.2 | 10 |
| Calibrator 5 | 15624 | 16122 | 15872 | 53.1 | 30 |
| Calibrator 6 | 24156 | 24054 | 24105 | 80.6 | 120 |
| Calibrator 7 (optional) | 25613 | 25217 | 25414 | 85.0 | 300 |
| Control I | --- | --- | --- | --- | --- |
| Control II | --- | --- | --- | --- | --- |
| Donor 1 | 20117 | 20080 | 20099 | 67.2 | 53 |

$$\text{Calculation of donor sample 1: } \frac{B}{T} (\%) = \frac{20099}{29914} \times 100 = 67\%$$

6 LIMITATIONS OF THE METHOD

Healthy individuals should be tested negative by using the Anti-GAD₆₅ RIA.

However, GAD₆₅ autoantibodies may be also present in a rare neurological disorder, Stiff-man Syndrome (SMS). Around 60 % of donors with SMS have GAD₆₅ autoantibodies in their serum. GAD₆₅ autoantibodies from donors with SMS have higher titers compared with those of donors with type 1 diabetes. That's why sera from donors with suspicion of SMS should be pre-dilute 1:50 and 1:100 with GAD₆₅ autoantibody negative sera. In donors with SMS GAD₆₅ autoantibodies occur also in cerebrospinal fluid.



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7 ANTI-GAD₆₅ ASSAY SCHEME

| | | | | | |
|----|---|---|----------|--------------------------|-------|
| 1 | Label test tubes* | CAL 1 - 6 (7) | CI - CII | Donor.-Sera 1, 2 etc. | T |
| 2 | Pipette Calibrators 1 - 6 (7) Control sera I - II Donor sera | 20 µl | 20 µl | 20 µl | |
| 3 | Pipette Tracer (made from D and J) | 50 µl | 50 µl | 50 µl | 50 µl |
| 4 | Incubate** | 2 hours (at room temperature) | | | |
| 5 | Pipette Protein A-Suspension (made from J and L) | 50 µl | 50 µl | 50 µl | |
| 6 | Incubate** | 1 hours (at room temperature) | | | |
| 7 | Pipette Buffer (J) | 1 ml | 1 ml | 1 ml | |
| 8 | Centrifuge | 20 minutes at 1500 x g | | | |
| 9 | Decant supernatant or Aspirate supernatant | leave tubes upside down on absorbent paper for 5 to 10 minutes quantitatively | | | |
| 10 | Count radioactivity | Counting time: 1 minute | | | |

* use conical tubes

** Prior to incubation, agitate the tubes briefly in order to ensure homogeneous reaction conditions.



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8 SAFETY PRECAUTIONS

- This kit is for research use only. Follow the working instructions carefully.
This instruction manual is valid only for the present kit with the given composition. An exchange of single components is not in agreement with CE regulations.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts (< 0.1 %) of sodium azide as a preservative. They must not be swallowed or allowed to come into contact with skin or mucosa. The possible formation of heavy metal azides in the drainage has to be prevented by sufficient rinsing with water.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for both Hepatitis an HIV antibody. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all donor samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
 - Do not smoke, eat or drink while handling radioactive material in any room designated for working with radioactive material,
 - Always use protective gloves,
 - Never pipette radioactive material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant,
 - Place contaminated tissues, tubes, bench covers, gloves etc. in a specially marked container, discard liquid and solid radioactive waste only as permitted by federal, state or local authorities and regulations.
- It is the responsibility of the user of this product to handle radioactive material in accordance to the national rules given by law or other statements of the local authorities.
- In any case GLP with all general and individual regulations has to be applied to the use of this kit.

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