



Revised 5 Dec. 2011 rm (Vers. 4.1)

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.

INTENDED USE

The 21-OH autoantibody RIA assay kit is intended for use by professional persons only, for measurement of 21-OH autoantibodies (21-OH Ab) in human serum.

REFERENCES

- H. Tanaka et al “Steroid 21-Hydroxylase Autoantibodies: Measurements with a New Immunoprecipitation Assay”
J. Clin. Endocrinol. Metab. 1997 82: 1440-1446
- J. Furmaniak and B. Rees Smith “Editorial: Adrenal and Gonadal Autoimmune Diseases”
J. Clin. Endocrinol. Metab. 1995 80: 1502 – 1505
- S. Chen et al “Autoantibodies to Steroidogenic Enzymes in Autoimmune Polyglandular Syndrome, Addison’s Disease, and Premature Ovarian Failure”
J. Clin. Endocrinol. Metab. 1996 81: 1871-1876
- G. Coco et al “Estimated Risk for Developing Autoimmune Addison’s Disease in Patients with Adrenal Cortex Autoantibodies”
J. Clin. Endocrinol. Metab. 2006 91: 1637-1645

Assay Principle

In 21-OH autoantibody radioimmunoassay (RIA) 21-OH antibodies in specimen sera, calibrators and controls are allowed to interact with highly purified recombinant ¹²⁵I 21-OH. After an overnight incubation solid phase protein A is added to precipitate antibody bound labelled 21-OH. Assay buffer is then added and the mixtures centrifuged. Any unbound ¹²⁵I 21-OH is removed from the tubes by aspiration of the supernatants. The level of radioactivity remaining in the tube is proportional to the antibody level in the test sample.

STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analyzed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C. 40 µL is sufficient for one assay (duplicate 20µL determinations). Repeated freeze thawing or increases in storage temperature must be avoided.

Do not use lipaemic or haemolysed serum samples.

When required, thaw test sera at room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to assay (preferably for 5 min at 10-15,000 x g in a microfuge) to remove particulate matter.

Please do not omit this centrifugation step if sera are cloudy or contain particulates.



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MATERIALS SUPPLIED IN 50 AND 100 TUBE KITS

MATERIAL	50 Tubes	100 Tubes
¹²⁵ I 21-OH	2 x 1.3 mL	4 x 1.3 mL
Calibrators	6 x 0.15 mL	6 x 0.15 mL
Controls (CONTROL +), (CONROL -)	2 x 0.15 ml	2 x 0.15 ml
Protein A	1 x 2.6 mL	2 x 2.6 mL
Assay buffer	1 x 120 mL	1 x 120 mL

Materials required but not Supplied

3 mL assay tubes

Pipettes capable of dispensing 20 µL, 50 µL 1 mL, 1.3 mL and 2.6 mL.

Vortex mixer

Refrigerated centrifuges capable of 1500 x g

Gamma counter.

PREPARATION OF REAGENTS SUPPLIED

A	¹²⁵ I labelled 21-OH, 25kBq/vial (at manufacture) Lyophilised Reconstitute each vial by addition of 1.3 mL assay buffer (D) and mix gently to dissolve. Once reconstituted, store at 2 – 8°C and use on day of reconstitution.
B1-6	Calibrators 0, 1, 5, 50, 500, 5000 U/mL (arbitrary units) Ready for use
C1-2	Controls 1 and 2 Ready for use. See vial label for concentration range
D	Assay buffer Ready for use.
E	Protein A Lyophilised Prior to use, reconstitute each vial by the addition of 2.6 mL assay buffer (D) and mix on a vortex mixer. Mix thoroughly immediately before use to ensure uniform suspension. Once reconstituted store at 2 – 8°C for up to the shelf life of the kit.

Assay Procedure

1. Pipette 20 µL (in duplicate) of specimen sera, calibrators (B1-6) and controls (C1-2) into respectively labelled assay tubes.
2. Pipette 50 µL of ¹²⁵I 21-OH into each tube and into two additional empty tubes for total counts.



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3. Mix each tube on a vortex mixer; cover with a suitable cover and incubate overnight (16 – 20 hours) at 2 – 8°C.
4. After incubation, pipette, 50 µL of protein A (E) into each tube (excluding the two total count tubes).
5. Mix each tube on a vortex mixer; cover the tubes with a suitable cover and Incubate at 2 – 8°C for 1 hour.
6. After incubation, pipette 1 mL of assay buffer (D) into each tube (excluding the two total count tubes), mix on a vortex mixer and centrifuge at 1500 x g for 30 minutes at 4°C.
7. After centrifugation, aspirate the supernatants and count each tube (including total count tubes) for ¹²⁵I for 1 minute.

RESULT ANALYSIS

A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the % binding of the calibrators on the y-axis (linear scale). The 21-OH Autoantibody concentrations in specimen sera can then be read off the calibration curve. Other data reduction systems can be used.

TYPICAL RESULTS

(example only; not for use in calculation of actual results)

Calibrator u/mL	% ¹²⁵ I 21-OH bound	Conc. u/mL
Total counts	47353	
0	4.0	
1	6.4	
5	13.0	
50	25.8	
500	45.9	
5000	69.7	
Control 1	15.1	7.3
Control 2	32.4	91.6

Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 5mg/mL or intralipid up to 3000 mg/dL.

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish their own normal and pathological reference ranges for 21-OH Ab levels.

SAFETY CONSIDERATIONS

Follow the instructions carefully.

Observe expiry dates stated on the labels and the specified stability for reconstituted reagents.

Refer to Material Safety Data Sheet for more detailed safety information.

The kit contains radioactive material.

This kit contains ¹²⁵I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

Users should make themselves aware of, and observe, any national and local legislation and codes of practice governing the use, storage, transportation and disposal of radioactive materials. Avoid all actions likely to lead to ingestion.



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Avoid contact with skin and clothing.
 Wear protective clothing and, where appropriate, personal dosimeters.
 Radioactive materials should only be used by authorised personnel and in designated areas.
 Wash hands thoroughly after handling.
 Monitor hands and clothing before leaving the designated area.
 Materials of human origin used in the preparation of the kit have been tested and found non-reactive for HIV1 and 2 and HCV antibodies and HBsAg but should, none the less, be handled as potentially infectious. Wash hands thoroughly if contamination has occurred and before leaving the laboratory.
 Sterilise all potentially contaminated waste, including test specimens, before disposal.
 Materials of animal origin used in the preparation of the kit have been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
 Some components contain small quantities of sodium azide as preservative.
 With all kit components, avoid ingestion, inhalation, injection or contact with skin, eyes or clothing.
 Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.

ASSAY PLAN

Allow all reagents and samples to reach room temperature before use (excluding assay buffer)

Pipette:	20 µL calibrators, controls and specimen sera
Pipette:	50 µL ¹²⁵ I 21-OH into all tubes plus two additional empty tubes for total counts
Tubes:	Mix on vortex mixer and cover
Incubate:	Overnight (16 – 20 hours) at 2 – 8°C
Pipette:	50 µL of protein A into all tubes (excluding the two total count tubes)
Tubes:	Mix on vortex mixer and cover
Incubate:	1 hour at 2 – 8°C
Pipette:	1 mL assay diluent (excluding the two total count tubes)
Tubes:	Centrifuge at 1500 x g for 30 minutes at 4°C
Tubes:	Aspirate supernatants
Count tubes using gamma counter	

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