

C3a Des Arg [¹²⁵I] RIA KIT

(REF: RK-518)

Intended Use

The [¹²⁵I]C3a Des Arg RIA system provides direct determination of human Complement C3a des Arg in human plasma. For research use only, not for use in diagnostic procedures.

Principle of method

This assay is based on the competition between unlabelled C3a and C3a des Arg and a fixed quantity of [¹²⁵I]-labelled C3a des Arg for a limited number of binding sites on a specific antibody. With fixed amounts of antibody and radioactive ligand, the amount of radioactive ligand bound will be inversely proportional to the concentration of added non-radioactive ligand.

The antibody bound C3a fraction is then reacted with goat anti-rabbit second antibody reagent. Separation of the antibody bound fraction is effected by centrifugation followed by decantation of the supernatant.

Measurement of the radioactivity in the pellet enables the amount of labelled C3a des Arg in the bound fraction to be calculated. The concentration of unlabelled C3a and C3a des Arg in the unknown is then determined by interpolation from a calibration curve. The calibration curve and unknowns should be prepared simultaneously.

C3a des Arg can be assayed in the range of 20-500 ng/ml (1-25 ng/tube). Each kit contains materials sufficient for 150 determinations permitting the construction of one calibration curve and the assay of 67 unknowns in duplicate.

Contents of the kit

- 1 vial TRACER (8.0 ml), ready for use after thawing, containing < 190 kBq, 5.1 μCi [¹²⁵I]C3a des Arg in phosphate buffered saline containing gelatin and 0.01 % sodium azide. Store at -15°C to -30°C.
- 5 vials CALIBRATOR (5 x 0.9 ml), ready for use after thawing, containing (A-E) 25, 10, 5, 2.5, 1 ng/ 50 μl human C3a des Arg in buffer with 0.01% sodium azide. Store at -15°C to -30°C.
- 1 vial ANTISERUM (8.0 ml), ready for use after thawing, containing rabbit anti-C3a des Arg antiserum solution in phosphate buffered saline with porcine gelatin and 0.01% sodium azide. Store at -15°C to -30°C.
- 1 vial ASSAY BUFFER (9.0 ml), ready for use, containing gelatin and 0.01 % sodium azide. Store at 2°C to 8°C after thawing.
- 1 vial SECOND ANTIBODY (8.0 ml), ready for use after thawing, containing goat anti-rabbit antiserum solution in phosphate buffered saline with gelatin and 0.01% sodium azide. Store at -15°C to -30°C.
- 1 bottle PRECIPITATING REAGENT (80 ml), ready for use, diluted in distilled water. Store at 2°C to 8°C after thawing.

Materials, tools and equipment required

Pipettes or pipetting equipment with disposable tips (50, 100, 450 and 2000 μl);

disposable polypropylene, polystyrene or glass tubes (12 x 75 mm) or conical shaped test tubes capable of withstanding centrifugation speeds up to 5000 xg (for a microfuge, 1.5 ml conical tubes are acceptable); test tube rack; isotonic saline solution; vortex mixer; plastic foil; adsorbent tissue; gamma counter.

Collection and Handling of Unknowns

Both the classical and alternative pathways of complement are known to be highly unstable in vitro. As a result, collection and storage of unknowns are critical.

Unknowns can be collected either in EDTA tubes or in collection tubes containing Futhan. During collection of unknowns using EDTA-Futhan tubes, all operations may be carried out at room temperature. Unknowns are stable for up to 3 hours prior to, and up to 24 hours after separation at room temperature.

Blood unknowns should be separated by centrifugation at 2000 xg for 15 minutes at 4°C. Plasma should be stored in single use aliquots at -15°C to -30°C. 0.45 ml plasma is required for assay.

Note: When processing blood unknowns, it is important to ensure that the solid EDTA-Futhan mixture completely dissolves. This can be achieved by gently mixing the contents by inverting the tube several times. It is important that unknowns collected in tubes without Futhan be separated and frozen within thirty minutes. Also frozen plasma unknowns collected in tubes without Futhan have limited stability.

Preparation of Unknowns

1. Label 2 sets of 12 x 75 mm disposable tubes. These tubes should be capable of withstanding centrifugation speeds of up to 5000 xg.
2. Pipette 450 μl of each unknown into one of the sets of labelled tubes (tubes dedicated for unknowns).
3. Pipette 450 μl precipitating reagent into each tube. (This creates a 1:1 dilution of the unknown).
4. Mix each tube well.
5. Incubate for 5-30 minutes at room temperature.
6. Centrifuge at a minimum of 2500 xg for 15 minutes at 4°C. (Microcentrifuges should not be used unless they are refrigerated).
7. Transfer the supernatants to the second set of labelled tubes. Discard the first tube. The supernatant should be processed immediately, not stored for later use.

Note: If, prior to the assay, it is anticipated that C3a concentration of unknown will lie above the upper limit of the assay range, it is recommended that the following dilution procedure be performed.

Assays can then be performed on both diluted and undiluted unknowns:

Dilute by mixing the supernatant in step 7 with isotonic saline containing disodium EDTA and Futhan. The most convenient method for preparation of the diluent is to add isotonic saline to a collection tube specified in the previous section. The resultant solution is then used as the diluent. Investigations have shown that unknowns containing elevated

levels of C3a des Arg can be diluted a maximum of 1:30 after addition of the precipitation reagent.

Preparation of reagents, storage

Storage: *see Contents of the kit.* At these temperatures each reagent is stable until expiry date. The actual expiry date is given on the package label and on the quality certificate.

Note: Assay components may be subjected to a maximum of two freeze-thaw cycles after initial use.

CAUTION!

Equilibrate all reagents and unknowns to room temperature prior to use.

Mix all reagents and unknowns thoroughly prior to use except for calibrators. Calibrators should be given a brief gentle mix only.

Assay procedure

(For a quick guide, refer to Table 1.)

1. Label 12x75 mm disposable tubes in duplicate for each total count (TC), non-specific binding (NSB), Bo (0 calibrator), calibrators (A-E) and each unknown.
2. Pipette 50 μl assay buffer into all tubes.
3. Vortex mix all tubes well to coat tube walls with buffer.
4. Pipette 50 μl isotonic saline into Bo tubes and 100 μl isotonic saline into NSB tubes.
5. Pipette 50 μl of each calibrator (SA-E) directly into the bottom of appropriately labelled tubes.
6. Vortex mix each treated plasma unknown (*see step 7 of Preparation of Unknowns*). Pipette 50 μl of each unknown (M_x) directly into the bottom of appropriately labeled tubes.
7. Pipette 50 μl of assay tracer into all tubes. The TC tubes should be stoppered and set aside for counting.
8. Pipette 50 μl of antiserum into all tubes except TC and NSB.
9. Vortex mix all tubes thoroughly. Cover the tubes, for example with plastic film, and incubate for 30 minutes at room temperature (15-30 °C).
10. Pipette 50 μl of goat anti-rabbit second antibody into all tubes except TC. Vortex mix and incubate for 30 minutes at room temperature.
11. Add 2 ml of isotonic saline to all tubes except TC.
12. Centrifuge all tubes together at 2000 xg for 10 minutes at 4 °C.
13. Carefully decant all tubes (except TC), discarding the supernatant. Keeping the tubes inverted, complete the removal of supernatant by standing the tubes on a pad of absorbent paper. Take care not to disturb the pellet while the tubes are inverted.
14. Count each tube for at least 60 seconds in a gamma scintillation counter (or for sufficient time to accumulate >10000 counts in the Bo tubes).
15. Calculate the C3a des Arg concentrations of the unknowns as described in the 'results' section of this protocol or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes are in microlitres)

Tubes	TC	NSB	Bo	Calibrator	Unknown
Buffer	50	50	50	50	50
Vortex mix					
Isotonic saline	-	100	50	-	-
Standard	-	-	-	50	-
Unknown	-	-	-	-	50
Tracer	50	50	50	50	50
Anti-serum	-	-	50	50	50
Vortex mix, cover tubes and incubate for 30 minutes at room temperature (15-30 °C)					
Second antibody	-	50	50	50	50
Vortex mix and incubate for 30 minutes at room temperature					
Isotonic saline	-	2000	2000	2000	2000
Centrifuge at 2000 xg for 10 minutes (4 °C), decant tubes and blot on filter paper					
Count radioactivity (60 sec/tube)					
Calculate the results					

Results

Calculate the average count per minute (CPM) for each pair of assay tubes. Subtract the average NSB cpm from all tubes except TC. If the counter background is high, it should be subtracted from all counts. Calculate the percent Bo/TC using the following equation:

$$\text{Bo/TC(\%)} = \frac{\text{Bo (cpm)} - \text{NSB (cpm)}}{\text{TC (cpm)}} \times 100$$

The average Bo/TC(%) should be in the range 30–60%. Calculate the percent bound for each calibrator and unknown using the following relationship:

$$\text{B/Bo(\%)} = \frac{\text{SA-E/M}_x \text{ (cpm)} - \text{NSB (cpm)}}{\text{Bo (cpm)} - \text{NSB (cpm)}} \times 100$$

A calibration curve can be generated by plotting the percent B/Bo as a function of the log C3a des Arg concentration.

Plot B/Bo(%) (y-axis) against concentration (ng) C3a des Arg per tube (x-axis). The concentration (ng per tube) value of the unknowns can be read directly from the graph (see table 2).

The concentration ng per tube value can be converted to ng/ml by multiplying by 40 as described below:

$$\text{ng/tube} \times 2 \text{ (1:2 dilution, step 3)} \times 20 \text{ (50 microlitres volume of unknown)} = \text{ng/ml}$$

Note: If unknowns were further diluted, this dilution factor must be taken into account.

Note: Curve-fitting algorithms available for data reduction may be used. It should be noted that whilst results may differ by up to 5–10% depending on the algorithm used, this should not affect assay precision.

Characterization of assay

Calibration

The assay calibrators are prepared from highly purified human C3a des Arg. The assay system is calibrated against a reference calibrator prepared from purified C3a des Arg.

Stability of Unknowns

The high sensitivity of RIA for complement activation products such as C3a means that *in vitro* complement activation in the unknown must be kept to a minimum. With this in mind, the stability of unknowns during collection, processing and storage in tubes containing disodium EDTA and the serine protease inhibitor, Futhan, and disodium EDTA alone, has been investigated. The results are shown below:

Unknown	EDTA+Futhan	EDTA only
Mean (ng/ml)	157	283
Std dev	27	104

These results were obtained from 50 blood unknowns collected into tubes containing EDTA+Futhan, and EDTA only. (It is recommended that individual laboratories determine levels for their own reference population).

Specificity

The antiserum cross-reactivities with related peptides are shown below:

Compound	Cross-reactivity (%)
C3	100
C3a	100
C3a des Arg	100
C4a des Arg	0.89
C5a des Arg	<0.22

Note: The assay procedure is selective for C3a and C3a des Arg, eliminating interference from C3.

Dilution

Unknowns may be diluted up to 1:30 with isotonic saline containing Futhan without affecting the accuracy of determination. The diluent is prepared by adding isotonic saline to an evacuated collection tube containing Futhan.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Do not use lipaemic, haemolyzed or turbid unknowns. The assay system is designed for use with collection tubes containing Futhan.

Warning

For research use only, not for use in diagnostic procedures.

Precautions

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to

the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human subjects. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1) and Hepatitis B surface Antigen (HBsAg). Care should always be taken when handling human biologicals to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other infectious agents are absent. Human blood should therefore be handled as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 3.8 mg.

Safety data sheet

Product name:

Sodium azide

CAS No. 26628-22-8

R: 22-32 Toxic if swallowed. Contact with acids liberates very toxic gas.

S: (1/2)-28-45 (Keep locked up and out of the reach of children). After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Composition:

Sodium azide solution.

Hazards identification:

Toxic if swallowed, inhaled, or absorbed through skin. May cause eye and skin irritation.

First aid measures:

In case of contact, immediately flush eyes or skin with copious amounts of water. If inhaled remove to fresh air. In severe cases seek medical attention.

Fire fighting measures:

Dry chemical powder. Do not use water.

Accidental release:

Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Mop up spill area, place waste in a bag and hold for waste disposal. Wash spill site area after material pick-up is complete.

Handling and storage:

Wear suitable protective clothing including overalls, safety glasses and gloves. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling.

Personal protection:

See above instructions for handling and storage.

Physical and chemical properties:

Formula weight: 65.01. Density: 1.850.

Stability and reactivity:

Avoid contact with metals and acid chlorides. This yields a very toxic gas.

Toxicological information:

LD50: 27 mg/kg oral, rat
LD50: 20 mg/kg skin, rabbit

Ecological information:

Not applicable

Disposal consideration:

Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.








Note: Inorganic azides will react with lead and copper plumbing fixtures to give explosive residues. Disposal of significant quantities of azides via such plumbing is not recommended.

Transport information :

No special considerations applicable.

Regulatory information:

The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

	Use by	AS	Antiserum
LOT	Batch code	CAL	Calibrator
	Caution, consult accompanying documents	SORB	Second antibody
	Biological risk	TRAC	Tracer
	Consult operating instructions	BUF	Assay buffer
REF	Catalogue number	REAG	Precipitating reagent
	Manufacturer		Temperature limitation Store at -15°C to -30°C
	Radioactive Material		

Website: <http://www.izotop.hu>

Technical e-mail: immuno@izotop.hu

Commercial e-mail: commerce@izotop.hu

IZOTOP

INSTITUTE OF ISOTOPES Ltd.

1535 Budapest. Pf.: 851.

Tel.: (36-1)392-2577, Fax: (36-1)395-9247

For ordering information, please contact:



Immuno-Biological Laboratories, Inc. (IBL-America)

8201 Central Ave NE, Suite P

Minneapolis, MN 55432

Toll Free: (888) 523-1246

Fax: (763) 780-2988

www.ibl-america.com

info@ibl-america.com