HUMAN C1 INACTIVATOR FUNCTIONAL ACTIVITY BINDARID™ KIT

For *in vitro* diagnostic use only

Product Code: RC002.3

Product manufactured by:
The Binding Site Group Ltd., 8 Calthorpe Road, Edgbaston, Birmingham, B15 1QT, UK.
www.bindingsite.co.uk
Telephone: +44 (0)121 456 9500
Fax: +44 (0)121 456 9749
e-mail: info@bindingsite.co.uk

CE

1 INTENDED USE

This kit is intended for assessing C1 inactivator activity in citrated human plasma.

2 SUMMARY AND EXPLANATION

The first complement component C1 is a macromolecule composed of three sub-components, C1q, C1r and C1s. Upon activation of the complement cascade the binding of C1 inactivator to activated C1r prevents further proteolysis of complement C4 by C1.

Deficiency of functional C1 inactivator is associated with hereditary angioneurotic oedma. In such individuals, C1 inactivator concentrations may be up to 20% of normal, but with no activity (ref. 1).

Radial immunodiffusion (RID) is a technique that is routinely used for measuring the concentration of various soluble antigens in biological fluids. It is principally derived from the work of Fahey & McKelvey (ref. 2) and Mancini et al. (refs. 3 & 4). In this kit, it is the quality of the precipitin rings that is evaluated (see below).

3 PRINCIPLE OF THE ASSAY

The method involves comparing C1r precipitation made by radial immunodiffusion on native plasma samples, and the same samples treated by adding heat-aggregated IgG. This causes the activation of the complement cascade and thus leads to binding of C1 inactivator to activated C1r.

The samples are applied to RID plates and allowed to diffuse radially from a cylindrical well through an agarose gel containing monospecific antibodies to human C1r. Antibody-antigen complexes are formed which, under appropriate conditions, will form a precipitation ring. By forming a complex with C1r the C1 inactivator effectively masks many of the antigenic sites of the C1r molecule, leading to deterioration in ring quality.

Samples containing functionally inactive C1 inactivator will show no such loss of ring quality.

4 REAGENTS

4.1 RID plates (supplied in resealable foil pouches). These contain monospecific sheep polyclonal antibodies to human C1r in agarose gel. Up to seven samples can be run per plate including normal and abnormal controls. Preservatives: 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA), 0.01% benzamidine.
4.2 Normal Control(s) Supplied in lyophilised form. Preservatives: 0.099% sodium azide, 0.1% EACA, 0.01% benzamidine.
4.3 Abnormal Control(s) Supplied in lyophilised form. Preservatives: 0.099% sodium azide, 0.1% EACA, 0.01% benzamidine.
4.4 Heat Aggregated IgG Supplied in lyophilised form. Preservatives: 0.099% sodium azide.

5 CAUTION

All donors of human plasma supplied in this kit have been tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either approved by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times.

Reagents from different batch numbers of kits are NOT interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kits should be stored at 2-8°C and can be used until the expiry date given on the kit box label. DO NOT FREEZE. The expiry dates of individual components are given on the component labels. RID plates should be stored at 2-8°C and are damaged by temperature extremes. Freezing will destroy the gel, therefore RID plates should be kept away from cooling elements in refrigerators. High temperatures should also be avoided as this will result in moisture loss from the gel, affecting performance. Unopened plates should be stored flat and upside down (pouch label uppermost) to prevent condensation accumulating in the wells. Handle plates with care to prevent gel damage.

Unopened controls and heat-aggregated IgG should be stored at 2-8°C. Once opened they are stable for at least one week at 2-8°C, but for longer storage they should be aliquoted and frozen (-20°C or below). All other reagents should be stored at 2-8°C.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen (-20°C or below) citrated plasma samples. Microbially contaminated haemolysed and very lipaemic plasma or those samples containing particulate matter should not be used. Blood samples should be collected by venepuncture and the plasma separated as soon as possible to prevent haemolysis. The plasma may be stored at 2-8°C for up to 48 hours prior to assay, or for prolonged storage, aliquoted and kept at -20°C or below. Repeated freezing and thawing should be avoided.

8 METHODOLOGY

A summary of the entire procedure is given at the end of this instruction leaflet

8.1 Materials provided

8.1.1 3 x *Human C1 Inactivator F.A. Bindarid* (radial immunodiffusion plates in foil pouches)
8.1.2 8 x *Gel Dividers*
8.1.3 2 x *Human C1 Inactivator F.A. Normal Control* (lyophilised)
8.1.4 2 x *Human C1 Inactivator F.A. Abnormal Control* (lyophilised)
8.1.5 4 x *Heat Aggregated Gamma Globulin* (lyophilised)
8.1.6 1 x instruction leaflet

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples, eg sample tubes, centrifuge etc.
8.2.2 Pipettes for accurate dilution of samples and controls.
8.2.3 Micropipettes for sample application. These should be capable of accurately delivering 10µL volumes. Binding Site Micropipettes (code AD041) or 'Hamilton' syringes are recommended.
8.2.4 Jewellers' Eyepiece (Code AD040) for magnifying and accurately assessing the precipitin ring intensity.
8.2.5 Distilled water for reconstitution of lyophilised components.
8.2.6 Physiological saline (0.9%/0.15M) for dilution of samples and controls.
8.2.7 37°C incubator.

8.3 Reagent preparation

8.3.1 RID Plate(s)

To avoid contamination of the gel, plates should be used in a dust-free environment. Take the plate from the foil pouch and remove the lid. If condensation is visible the plate should be kept upside down until the lid has been removed to prevent droplets falling onto the gel. Check the plate to ensure that no damage has occurred in storage or transit, e.g. splits in the gel. Leave the plate open for 10-15 minutes (or longer if necessary) at room temperature to allow any condensation in the wells or on the gel surface to evaporate. Samples should never be applied to wells in which moisture is still visible.

Plate partitioning: The plates may be partitioned into up to four sections using the gel dividers provided prior to use. Each divider should be positioned carefully on the gel, cutting edge downward, with the stabilising arm resting on the central plate label. Press firmly on the arm to cut the gel and leave in position.

Plate partitioning is recommended if only part of the plate is to be used initially or when measuring suspected high concentration samples which could (by diffusing over a wide area) result in antibody depletion occurring elsewhere on the plate. After initial use, partitioned plates should be resealed in their foil pouches and stored at 2-8°C with the gel divider(s) in place. Store partitioned plates right side up and use within four weeks.

8.3.2 Heat-aggregated IgG (HAG)

The HAG should be reconstituted using the volume of distilled water stated on the vial label.

8.3.3 Samples

Two aliquots of each sample should be taken. The first of these should be mixed with physiological saline in the ratio 3 parts sample to 1 part saline (eg mix 150µL sample with 50µL saline). The second aliquot should be mixed with HAG in the ratio 3 parts sample to 1 part HAG (eg 150µL sample with 50µL HAG).
Both aliquots should be incubated for 60 mins at 37°C before applying to the RID plate(s).

8.3.4 Controls

Both the normal and the abnormal controls should be reconstituted using the volume of distilled water stated on their vial labels. When completely dissolved they should be treated in the same way as patient samples (see section 8.3.3).

8.4 Application of calibrators and samples

The controls and test samples should be gently mixed immediately before use. Fill one well with 10µL of the normal control / saline mixture and one with the normal control/HAG mixture. Fill a third well with 10µL of the abnormal control / saline mixture and a fourth with the abnormal control/HAG mix. The remaining wells should be filled with sample / saline and sample/HAG mixes.

8.5 Incubation

After sample application the lid is tightly closed and the plate stored flat at room temperature (approximately 20-24°C). It is essential that the gel is not allowed to dry out during incubation. To minimise evaporation, it is suggested that plates should either be resealed in their foil pouches or stored in a moist box (a sealed plastic box containing damp tissue paper) during incubation. The minimum recommended diffusion time is 96 hours. Final ring intensity may be affected by temperature. Extremes of temperature should be avoided.

8.6 Quality control

The control should be treated in the same way as patient samples. The normal control with HAG should show considerable deterioration in ring quality in comparison with the ring given by the normal control with saline.

In contrast, there should be no difference in ring quality between the differently treated aliquots of the abnormal control.

9 RING ASSESSMENT AND RESULT PROCESSING

9.1 Ring assessment

After the required diffusion time, ring intensity should be evaluated using the naked eye, a jeweller's eye piece. **It is most important to use bright side lighting, optimally in a darkened room and against a dark background.**
Note any difference in ring intensity, between samples and controls, see interpretation example below.

9.2 Interpretation

The diagram below gives a guide to the intensity of precipitin rings to be expected with 'normal' and C1 Inactivator functionally inactive samples.

Normal plasma		C1 Inactivator (functionally inactive) plasma	
3+1 Saline	3+1 HAG	3+1 Saline	3+1 HAG

Weak ring | Very weak/ not visible | Rings visible

10 LIMITATIONS OF PROCEDURE

10.1 TROUBLE SHOOTING

Problem	Possible Cause (s)	Suggested Action (s)
A. No ring for:		
1. Control(s)	Control omitted.	Repeat assay.
2. Test sample	Sample omitted.	Repeat assay.
3. Control(s) and test samples	Plate deterioration.	a) Storage damage. Repeat assay using new plate.
		b) Product expired. Repeat assay using new plate/kit.
B. Non-circular rings	i) Poor sample application.	Repeat assay.
	ii) Gel dried out before use.	a) Storage damage. Repeat assay using new plate.
		b) Product expired. Repeat assay using new plate/kit.
	iii) Gel dried out during sample application or incubation.	Repeat assay minimising the time the plate is left open. Incubate with lid on tight in a moist box or sealed pouch.
	iv) Local antibody depletion (due to high concentration samples on the plate).	Dilute samples and repeat assay.
C. Cloudy gel	i) Plate has been frozen.	Repeat assay using new plates. Review storage.
	ii) Gel dried out before use.	As for B (ii) above.
	iii) Gel dried out during sample application or incubation.	As for B (iii) above.
D. Weak, pitted gel	Plate has been frozen.	Repeat using new plate. Review storage.
E. Double/Multiple rings	Non-specific precipitation close to well (due to PEG in gel).	Read outer ring.

10.2 Diagnosis cannot be made and treatment must not be initiated on the basis of C1 Inactivator Funtional Activity alone. Clinical history and other laboratory findings must be taken into account.

10.3 If an unexpected result is obtained, the assay should be repeated, preferably with a fresh sample.

If a problem cannot be resolved, please refer to supplier.

11 BIBLIOGRAPHY

1. Zicarrdi R J Cooper N R (1978). Modulation of the Antigenicity of C1r and C1s by C1 Inactivator. J. Immunol., **121**, 2148-2152.
2. Fahey, J L & McKelvey, E M (1965). Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immunol., **94**, 84-90.
3. Mancini, G, Vaerman, J P *et al* (1964). Protides of the biological fluids (XI Colloquium). Peters H. (ed), Publ. Elsevier Publishing Co., Amsterdam p370.
4. Mancini, G, Carbonar, A O *et al* (1965). Immunodiffusion. Immunochem, **2**, 235-254.

12 SUMMARY OF PROCEDURE

12.1 Take two aliquots of each sample or control.
12.2 Treat one aliquot of each with heat aggregated IgG, by mixing 150µL of sample with 50µL of heat aggregated IgG.
12.3 Treat the second aliquot of each with physiological saline by mixing 150µL of sample with 50µL of physiological saline solution.
12.4 Incubate samples and controls at 37°C for 1 hour.
12.5 Allow condensation to evaporate from RID plate(s).
12.6 Apply controls and samples to RID plate(s) in 10µL volumes.
12.7 Replace lid and incubate at room temperature (approximately 20-24°C) for 96 hours.
12.8 Compare ring intensities between differently treated aliquots of the same samples and controls.