



**\*\*REPRESENTATIVE DATASHEET\*\***

## Matched-Pair Antibody Set For Neutralization of human Heparin Cofactor II (HCII) in Plasma

Sufficient reagent for 10 determinations

**Product #:** HCII-NU10  
**Lot #:** XXXX  
**Expiry Date:** XXXX

Store at 2 -8°C

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For Research Use Only  
Not for use in diagnostic procedures.

### Description of Heparin Cofactor II

Heparin Cofactor II (HCII), also known as heparin cofactor A and dermatan sulphate cofactor, is a single chain glycoprotein produced in the liver and circulates in plasma at concentration of 50-100 µg/ml (0.8-1.5 µM). A member of the SERPIN family of proteinase inhibitors, HCII exhibits a relatively narrow specificity and is only known to inhibit the serine proteinases thrombin and chymotrypsin. The inhibitory activity toward thrombin is accelerated up to 1000-fold in the presence of optimal concentrations of heparin. Unlike Antithrombin, thrombin inhibition by HCII is also enhanced by dermatan sulphate. HCII has an apparent molecular weight of 65,600 daltons and interaction with thrombin results in a covalent 1:1 enzyme-inhibitor complex of 102,600 daltons<sup>1-3</sup>.

### Principle of HCII Neutralization

Polyclonal sheep antibody to human HCII is added directly to human plasma and allowed to incubate for 20 minutes. During this time the antibody will bind to and inactivate (neutralize) the ability of HCII to inhibit thrombin in the presence of dermatan sulphate. Clot-based tests can then be performed on the neutralized sample. To control for the addition of antibody volume and buffer, a control antibody (non-immune sheep IgG, at the same concentration and in the same buffer) is added separately to a duplicate sample and the test is repeated.

### References:

1. Tollefsen DM, Majerus DW, Blank MK; Heparin Cofactor II; JBC 257:5, p2162-2169, 1982.
2. Pratt CW, Whinna HC, Mead JB, Treanor RE, Church FC: Physicochemical aspects of Heparin Cofactor II; Ann N.Y. Acad. Sci.: 556, p104-115, 1989.
3. Griffith MJ, Noyes CM, Church FC; Reactive Site Peptide Structural Similarity between Heparin Cofactor II and Antithrombin III; JBC:260, pp 2218-2225, 1985.

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### Product Description:

**1. Neutralizing IgG (HCII-NU10-T):** One purple-capped vial containing 0.1 ml of sheep antibody (IgG) to human HCII.

**2. Control IgG (HCII-NU10-C):** One white-capped vial containing 0.1 ml of sheep IgG prepared from non-immune serum for use as a negative control.

**Buffer:** Both the neutralizing and control IgG have the same protein concentration in 0.02 M HEPES, pH 7.4, 0.01 M Na<sub>3</sub>-Citrate, 0.15 M NaCl and 0.02% (w/v) NaN<sub>3</sub>.

**Storage:** Refrigerate at 2-8°C

### Test Procedure:

1. Prepare duplicate test tubes, each containing 90 µL of plasma.
2. To a tube labelled test, add 10 µL of test IgG (HCII-NU10-T).
3. To a tube labelled control, add 10 µL of control IgG (HCII-NU10-C)
4. Mix contents of both tubes and incubate at room temperature for 20 minutes. Both plasmas may be used directly or placed on ice for up to 4 hours.

In the quality control of this product, Thrombin Clot Times were performed as follows:

**5 µL** of 2.5 mg/mL dermatan sulphate (final =0.25 mg/mL in plasma)

**50 µL** of normal citrated plasma

**150 µL** of Seegers solution (28 mM Imidazole, 1.7 % (w/v) acacia, 3.7 mM CaCl<sub>2</sub>, 57 mM NaCl, pH 7.3)

**50 µL** of human thrombin, adjusted to produce a clot time of approximately 30 seconds in the absence of heparin

Clot times were measured using the ST-4 BIO analyzer (Diagnostica Stago) according to manufacturer's directions.

### Technical Notes:

Under the conditions described above, greater than 99% of HCII in normal plasma is neutralized. This is confirmed using HCII deficient plasma (product # HCII-DP).

The above conditions are only intended for use in screening assays of compounds that may exert anticoagulant activity through HCII. The use of these reagents for quantitative determination of HCII activity is not recommended.

### Related Products:

Cat #:	<b>GAHC2-IG</b>	Goat anti- HCII, whole IgG from antiserum
Cat #:	<b>SAHC2-APHRP</b>	Goat anti-HCII, APiGg-peroxidase conjugated
Cat. #	<b>SAHC2-IG</b>	Sheep anti- HCII, whole IgG from antiserum
Cat #:	<b>HCII-EIA</b>	Paired antibody set for ELISA of HCII, 4 x 96 wells
Cat #:	<b>THCII-EIA</b>	Paired antibody ELISA of Thrombin-HCII complexes
Cat #:	<b>HCII-DP</b>	Human plasma deficient in HCII, immune depleted
Cat #:	<b>ATHC-DP</b>	Human ATIII & HCII def plasma, immune depleted

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