

AIDA
autoimmune diagnostic assays

REF 10291
HiT II Check



Instruction Manual

Table of Contents

1	Intended Use	1
2	Clinical Application and Principle of the Assay	1
3	Kit Contents	2
4	Storage and Shelf Life	2
5	Precautions of Use.....	3
6	Sample Collection, Handling and Storage.....	4
7	Assay Procedure	4
8	Quantitative Interpretation.....	7
9	Technical Data.....	8
10	Performance Data.....	8
11	Literature	9



	Product Ref.	10291
	Product Desc.	HiT II Check
	Manual Rev. No.	001 : 2017-09-26

1 Intended Use

HiT II Check is a solid phase enzyme immunoassay for the quantitative detection of antibodies that cause heparin induced thrombocytopenia type II.

2 Clinical Application and Principle of the Assay

Heparin induced thrombocytopenia (HiT) is a severe side-effect of heparin treatment and occurs in 1-3 % of the treated patients.

Two distinct types of heparin induced thrombocytopenia can occur: HiT type I has no clinical relevance and is characterised by a transient decrease in platelet counts. It recovers after a few days even if the administration of heparin continues. HiT type II is an immune-mediated form. The platelet counts drop more than 50 % from the baseline 5 to 14 days after first beginning of heparin administration. The affected patients develop antibodies that recognize neoepitopes exposed by the complex of platelet factor 4 and heparin. The antibodies found are most commonly of the IgG subclass with or without IgM and IgA class antibodies. IgM and IgA are rarely found without IgG antibodies. However, the pathogenic effect has been proofed for the IgG subclass whereas the effect of IgM and IgA antibodies is still controversially discussed. The Fc portion of the HiT antibody can bind to the platelet Fc receptor and this interaction triggers activation and aggregation of the platelets. Activated platelets release PF4, thus perpetuating the cycle of heparin-induced platelet activation. The platelet activation leads to the production of platelet factor 4 and prothrombotic platelet microparticles. Heparin-like molecules (heparan sulfate) on the surface of endothelial cells are also able to form a complex with platelet factor 4 that can be recognized by HiT antibodies. That, in turn, can induce tissue factor expression with further activation of the coagulation cascade and thrombin generation. It leads to an increased risk for new arterial and venous thromboembolic complications that can be lethal in 10-15 % of the patients.

An early diagnosis and a replacement with a suitable alternative anticoagulant can clearly minimize the complication rate.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

	Product Ref.	10291
	Product Desc.	HiT II Check
	Manual Rev. No.	001 : 2017-09-26

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgA/G/M	1 x 15ml	White	Red	Containing: Immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H ₂ O ₂)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

4 Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

 autoimmune diagnostic assays	Product Ref.	10291
	Product Desc.	HiT II Check
	Manual Rev. No.	001 : 2017-09-26

5 Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of the intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All biological source material used for some reagents of this kit has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle these as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

5.2 General directions for use

In case that the product information, including the labeling, is defective or incorrect please contact the manufacturer or the supplier of the test kit.

Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature ($20\text{-}32^\circ\text{C}/68\text{-}89.6^\circ\text{F}$) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at $30^\circ\text{C}/86^\circ\text{F}$ for automated systems.

Never expose components to higher temperature than $37^\circ\text{C}/98.6^\circ\text{F}$.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

	Product Ref.	10291
	Product Desc.	HiT II Check
	Manual Rev. No.	001 : 2017-09-26

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8h, respectively stored tightly closed at 2-8°C/35-46°F up to 48h, or frozen at -20°C/-4°F for longer periods. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4)

7 Assay Procedure

7.1 Preparations prior to starting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For *QUANTITATIVE* interpretation

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	...	
H	Cal D	NC	...	

CalA: calibrator A

CalB: calibrator B

CalC: calibrator C

CalD: calibrator D

CalE: calibrator E

CalF: calibrator F

PC: positive control

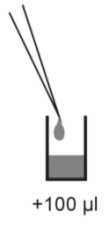
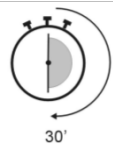
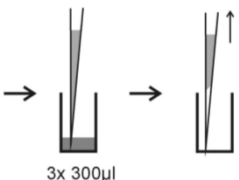
NC: negative control

P1: patient 1

P2: patient 2

P3: patient 3

7.3 Test Steps

Step	Description
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with quantitative interpretation results desired:
CONTROLS & SAMPLES	
3.	<div style="display: flex; align-items: center;">  <div style="margin-left: 20px;"> <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either:</p> <p style="text-align: center;">Calibrators (CAL.A to CAL.F) for <i>QUANTITATIVE</i> interp.</p> <p>and 100 µl of each of the following:</p> <ul style="list-style-type: none"> • Negative control (NC) and Positive control (PC), and • Patients diluted serum (P1, P2...) </div> </div>
4.	<div style="display: flex; align-items: center;">  <div style="margin-left: 20px;"> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p> </div> </div>
5.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;"> <p style="border: 1px solid black; padding: 2px;">WASHB</p>  </div> <div> <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p> </div> </div>

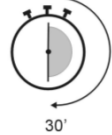
CONJUGATE

6. **CONJ**



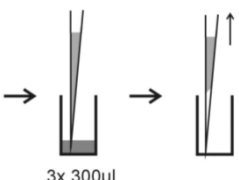
Pipette 100 µl conjugate into each well.

7.



Incubate for 30 minutes at 20-32°C/68-89.6°F.

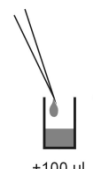
8. **WASHB**



Wash 3x with 300 µl washing buffer (diluted 1:50).

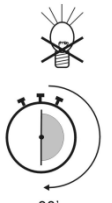
SUBSTRATE

9. **SUB**



Pipette 100 µl TMB substrate into each well.

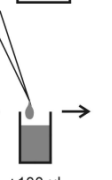
10.



Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.


STOP

11. **STOP**



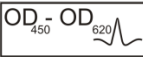
Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.

12.



Incubate 5 minutes minimum.

13. Agitate plate carefully for 5 sec.

14.  Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

450/620 nm

8 Quantitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 16 U/ml	16 - 24 U/ml	>24 U/ml

Example of a standard curve

Do NOT use this example for interpreting patient's result

Calibrators IgA/G/M	OD 450/620 nm	CV % (Variation)
0 U/ml	0,025	0,0
3 U/ml	0,139	3,5
10 U/ml	0,283	4,3
30 U/ml	0,598	4,0
100 U/ml	1,224	3,6
300 U/ml	2,123	2,8

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0,793/0,801	0,797	47,7
P 02	0,308/0,333	0,321	12,1

Do not use this example for interpreting patients results!

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

	Product Ref.	10291
	Product Desc.	HiT II Check
	Manual Rev. No.	001 : 2017-09-26

9 Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35-46°F use original vials only.
Number of determinations:	96 tests

10 Performance Data

10.1 Analytical sensitivity

Testing sample buffer 30 times on HiT II Check gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and sensitivity

The sensitivity of the HiT II Check was determined to be 100 % in comparison to plasma samples with known immune status. Clinically defined plasma samples show a specificity of 90%.

10.3 Linearity

Chosen plasma samples have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	Measured (U/ml)	Expected (U/ml)	Recovery (%)
1	1 / 100	77,6	80,0	97,0
	1 / 200	37,5	40,0	93,8
	1 / 400	18,8	20,0	94,0
	1 / 800	9,1	10,0	91,0
2	1 / 100	7,7	8,0	96,3
	1 / 200	3,8	4,0	95,0
	1 / 400	2,2	2,0	110,0
	1 / 800	1,0	1,0	100,0

	Product Ref.	10291
	Product Desc.	HiT II Check
	Manual Rev. No.	001 : 2017-09-26

10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three plasma samples selected to represent a range over the standard curve.

Intra-assay		
Sample No.	Mean (U/ml)	CV (%)
1	9,8	1,3
2	120,2	9,8
3	210,2	10,0

Inter-assay		
Sample No.	Mean (U/ml)	CV (%)
1	10,2	1,0
2	106,6	9,5
3	208,6	6,3

10.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11 Literature

Warkentin T.E. (2005). New approaches to the diagnosis of heparin induced thrombocytopenia. *Chest* 127: 35-45.

Franchini M. (2005). Heparin induced thrombocytopenia: an update. *Thrombosis Journal* 3: 14.

Warkentin T.E. (2004). Heparin induced thrombocytopenia. Diagnosis and treatment *Circulation* 110: 454-458.

Lindhoff-Last E., Gerdson F., Ackermann H., Bauersachs R. (2001). Determination of heparin-platelet factor 4-IgG antibodies improves diagnosis of heparin induced thrombocytopenia. *British Journal of Haematology* 113: 886-890.

Warkentin T.E., Chong B.H., Greinacher A. (1998). Heparin induced thrombocytopenia: Towards consensus. *Thrombosis and Haemostasis* 79: 1-7





Ziporen L., Li Z.Q., Park K.S., Sabnekar P., Liu W.Y., Arepally G., Shoenfeld Y., Kiebert-Emmons T., Cines D.B., Poncz M. (1998). Defining an antigenic epitope on platelet factor 4 associated with heparin-induced thrombocytopenia. *Blood* 92, 9: 3250-3259.

Greinacher A., Poetsch B., Amiral J., Dummel V., Eichner A., Mueller-Eckhardt C. (1994). Heparin-associated thrombocytopenia: isolation of the antibody and characterization of a multimolecular PF4-heparin complex as the major antigen. *Thrombosis and Haemostasis* 71: 247-251.

Chong B.H., Fawaz I., Chesterman C.N., Berndt M.C. (1989). Heparin induced thrombocytopenia: mechanism of interaction of the heparin-dependent antibody with platelets. *British Journal of Haematology* 73: 235-240.

Lothar Thomas: Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik., 8. Auflage, TH Books

CLSI Guideline GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

REF	" Numero d'ordine	" Catalogue number
	" Référence Catalogue	" Numéro de catálogo
	" Bestellnummer	" Αριθμός παραγγελίας
	" Número de catálogo	
LOT	" Descrizione lotto	" Lot
	" Lot	" Lote
	" Chargen Bezeichnung	" Χαρακτηρισμός παρτίδας
	" Lote	
	" 96 determinazioni	" 96 tests
	" 96 tests	" 96 pruebas
	" 96 Bestimmungen	" 96 προσδιορισμοί
	" 96 Testes	
	" Da utilizzarsi entro	" Use by
	" Utilise avant le	" Utilizar antes de
	" Verwendbar bis	" Χρήση μέχρι
	" Utilizar antes de	
	" Conservare a 2-8°C	" Store at 2-8°C (35-46°F)
	" Conserver à 2-8°C	" Conservar a 2-8°C
	" Lagerung bei 2-8°C	" Φυλάσσεται στους 2-8°C
	" Conservar entre 2-8°C	
	" Prodotto da	" Manufactured by
	" Fabriqué par	" Fabricado por
	" Hergestellt von	" Κατασκευάζεται από
	" Fabricado por	
CO-CAL	" Calibratore cut-off	" Cut off Calibrator
	" Etalon Seuil	" Calibrador de cut-off
	" Grenzwert Kalibrator	" Οριακός ορός Αντιδραστήριο βαθμονόμησης
	" Calibrador de cut-off	
CON +	" Controllo positivo	" Positive Control
	" Contrôle Positif	" Control Positivo
	" Positiv Kontrolle	" Θετικός ορός ελέγχου
	" Controllo positivo	
CON -	" Controllo negativo	" Negative Control
	" Contrôle Négatif	" Control Negativo
	" Negativ Kontrolle	" Αρνητικός ορός ελέγχου
	" Controllo negativo	
CAL	" Calibratore	" Calibrator
	" Etalon	" Calibrador
	" Kalibrator	" Αντιδραστήριο βαθμονόμησης
	" Calibrador	
RC	" Recupero	" Recovery
	" Corrélation	" Recuperado
	" Wiederfindung	" Ανάκτηση
	" Recuperação	
CONJ	" Coniugato	" Conjugate
	" Conjugé	" Conjugado
	" Konjugat	" Σύζευγμα
	" Conjugado	
MP	" Micropiastro rivestita	" Coated microtiter plate
	" Microplaque sensibilisée	" Microplaca sensibilizada
	" Beschichtete Mikrotiterplatte	" Επικαλυμμένη μικροπλάκα
	" Microplaca revestida	
WASHB 50x	" Tampone di lavaggio	" Wash buffer
	" Tampon de Lavage	" Solución de lavado
	" Waschpuffer	" Ρυθμιστικό διάλυμα πλύσης
	" Solução de lavagem	
SUB	" Tampone substrato	" Substrate buffer
	" Substrat	" Tampón sustrato
	" Substratpuffer	" Ρυθμιστικό διάλυμα υποστρώματος
	" Substrato	
STOP	" Reagente bloccante	" Stop solution
	" Solution d'Arrêt	" Solución de parada
	" Stopreagenz	" Αντιδραστήριο διακοπής αντίδρασης
	" Solução de paragem	
SB 5x	" Tampone campione	" Sample buffer
	" Tampon Echantillons	" Tampón Muestras
	" Probenpuffer	" Ρυθμιστικό διάλυμα δειγμάτων
	" Diluente de amostra	