IMPACT P2Y12 – For Research Use Only

REF **HB-2127-FG**

Guide to Symbols

REF	Catalogue No.	Σ	Contains sufficient for <n> tests
	Consult accompanying Documents	BIO	Contains biological material of animal origin
2℃ 8℃	Store at 2-8°C		Use By Date
LOT	Batch – Lot Code	RCNS 210µl Aqua	Reconstitute with 210µl De-ionised or Purified Water

IFU118-RUO Version 0

Hart Biologicals Ltd.
2 Rivergreen Business Centre
Queens Meadow,
Hartlepool, TS25 2DL. UK
Tel: +44 (0) 1429 271100 Fax: +44 (0) 1429 277085
www.hartbio.co.uk e-mail: sales@hartbio.co.uk

IMPACT P2Y12 – For Research Use Only

GB Handling Instructions

Section 1: Manufacturer
Hart Biologicals Ltd. 2 Rivergreen Business Centre, Queens Meadow, Hartlepool. UK. TS25 2DL.

Section 2: Identification of the Reagent
IMPACT P2Y12 – For Research Use Only available as:
6 x 210µl – HB-2127-FG

Section 3: Intended use
Hart Biologicals IMPACT P2Y12 – For Research Use Only is used as an antagonist for platelet aggregation testing in whole blood, during testing on the IMPACT analyser with IMPACT ADP – For Research Use Only. For use by trained professionals only.
IMPACT P2Y12 – For Research Use Only, comprises of Ticagrelor, which is an inhibitor for the P2Y12 receptor activation pathway of human platelets. The addition of IMPACT P2Y12 – For Research Use only, to the blood sample, blocks the P2Y12 receptor of platelets, reducing the aggregation response for ADP induced platelet aggregation. This inhibitor can be used to detect P2Y12 receptor defects or to simulate P2Y12 receptor blocker intake (positive control) in antiplatelet studies.[3,8,10]
IMPACT P2Y12 – For Research Use Only product contains a lyophilised preparation of 0.02mg/ml Ticagrelor with added buffer and stabilisers.

Section 4: Principles of the examination method
IMPACT utilises whole blood impedance aggregometry (WBIA), an established technology for platelet function testing that has been widely used for many years. It is a rapid and standardised option for platelet function testing. [1,2,9] The method is impedance (electrical resistance) aggregometry and is non optical.[5] The analyser can be used to test platelet function and for antiplatelet studies.[3,10] Test principle is that platelets adhere to small wires suspended in the blood sample and the impedance between the wires as the platelets adhere and aggregate is measured and plotted.[8] In the IMPACT aggregation trace, the impedance is measured numerically in ohms and displayed graphically and numerically on the screen. Two measurements are performed in parallel in each test cell to provide internal verification and assurance.
P2Y12 is a G-protein-coupled receptor that is activated upon ADP binding. The activation of the P2Y12 mediated signalling pathway leads to platelet aggregation. Ticagrelor is a well-known antiplatelet drug that inhibits P2Y12 receptors of platelets. Upon addition of the IMPACT P2Y12 – For Research Use Only, to the blood sample, the P2Y12 receptor of platelets is blocked and therefore ADP induced aggregation tests are inhibited, particularly IMPACT ADP – For Research Use Only, tests.[6,8]

Section 5: Traceability of values assigned to calibrators and trueness-control material
No control material is currently available for platelet aggregation testing. However, IMPACT Control Set – For Research Use Only, is provided for verification of the IMPACT analyser, with two level impedance quality control.

It is recommended that each laboratory establishes its own normal range parameters consisting of fresh whole blood samples collected from normal healthy donors.

IMPACT P2Y12 – For Research Use Only, is an inhibitor for the P2Y12 pathway of human platelets and can be used together with IMPACT ADP – For Research Use Only.

Section 6: Components
IMPACT P2Y12 – For Research Use Only is supplied as:
HB-2127-FG 6 x 210µl of lyophilised Ticagrelor (0.02mg/ml). Each kit is sufficient to perform 60 tests.

Section 7: Additional required equipment
IMPACT Operator's Manual - Research Use Only – IFU123-RUO
IMPACT Analyser – For Research Use Only (HB-2116-FG)
De-ionised or Purified Water
IMPACT Test Cells 5 x 12 – For Research Use Only (HB-2117-FG)
IMPACT Diluent – For Research Use Only (HB-2129-FG)
IMPACT Diluent + Calcium – For Research Use Only (HB-2130-FG)
IMPACT Control Set – For Research Use Only (HB-2131-FG)
IMPACT ADP – For Research Use Only (HB-2118-FG)

Section 8: Reagent preparation
Allow the IMPACT P2Y12 – For Research Use Only vial to come to room temperature and then reconstitute using the reconstitution setting within the IMPACT software with automated pipette volume. IMPACT Operators' Manual - Research Use Only – IFU123-RUO should be referred to.
Use purified water as indicated on the vial label. Allow the IMPACT P2Y12 – For Research Use Only vial to reconstitute at room temperature for 10 minutes. The IMPACT P2Y12 – For Research Use Only reagent is then ready to use.

Section 9: Storage and shelf life after first opening
Unopened vials (lyophilised):
- 3 years @ 2-8°C

Opened vials (after reconstitution):
- 7 Days @ 2-8°C
-24 Hours On Board @ Room Temperature (18-25°C)

The vial label carries the expiry date of the reagent from the date of manufacture.

Section 10: Warnings and precautions
For Research Use Only.
Contains animal derivatives:
Bovine Serum Albumin (BSA) (0.5%) used as a lyoprotectant within IMPACT P2Y12 – For Research Use Only, was prepared from bovine plasma, which was tested for the absence of infectious agents and collected from animals free from VSE and BT Virus. However, no test may exclude fully the absence of infectious agents. As with any product of bovine origin, it must be used with all cautions required for handling potentially hazardous material.

According to REACH (EC) No. 1907/2006 and CLP Regulation (EC) No. 1272/2008, IMPACT P2Y12 – For Research Use Only, product is not classifiable as hazardous.

IMPACT P2Y12 – For Research Use Only, vials, may be disposed of in accordance with laboratory procedures for hazardous waste. IMPACT P2Y12 – For Research Use Only Kit Packaging may be recycled in line with local regulatory requirements.

Section 11: Primary sample collection, handling and storage
The recommended sample collection for impedance aggregometry using whole blood is stated as follows: Citrated tubes (3.2 % Citrate) or Hirudin (25µg/ml) as anticoagulant may be used.[4,5,7] Guidelines for blood draw of Platelet Function Testing (PFT) should be followed with needle gauge of 19-21G.[4] It is only

possible to compare the results of an individual sample with reference ranges when the same sample anticoagulant (i.e. Citrate or Hirudin) is used.
Samples should be analysed within the period of 0.5-3 hours after blood collection. [5,7] Prior to immediate testing the sample, the blood tube should be gently inverted by hand. [5,6]

Section 12: Examination procedure
The IMPACT analyser is designed to ensure easy, efficient, and safe user handling. The Software User Interface is developed for the use of a touchscreen and semi-automated test programme with step by step protocol. IMPACT Operator's Manual - Research Use Only – IFU123-RUO, should be referred to.
Data entry can be performed via the integrated label scanner on the front of the IMPACT analyser or manually via an integrated keyboard.) Test volumes used during the IMPACT P2Y12 test protocol are stated as follows:

- 300µl IMPACT Diluent / IMPACT Diluent + Calcium
- 300µl Whole Blood Sample
- 20µl IMPACT P2Y12
- Incubation
- 20µl IMPACT ADP – to start the test
- Measuring time – 6 Minutes

Section 13: Control Procedure
For Research Use Only. The results of platelet aggregation studies should be interpreted against the results of aggregation profiles of a normal sample tested at the same time. The normal donor should not have taken acetylsalicylic acid or acetylsalicylic acid containing compounds COX inhibitors, or other platelet aggregation inhibitors within 10 days.
Each laboratory should define its acceptable ranges and verify the expected performance characteristics.
Results of IMPACT ADP – For Research Use Only should be compared to IMPACT P2Y12 – For Research Use Only.
IMPACT Control Set – For Research Use Only should be used to verify performance of the IMPACT analyser prior to testing samples.

Section 14: Calculation of examination results
During the IMPACT testing, results are expressed within the software interface as Area Under Curve (AUC) value, this is the measured impedance in ohms*minute. Additionally, the Maximum Slope in ohms/minute and the Maximum Aggregation in ohms are also displayed. These values should be recorded as well as the aggregation trace and considered alongside a normal whole blood sample control and inhibited control sample where applicable. Normal IMPACT ADP induced aggregation values are deemed 'normal' AUC >60 ohms*min. IMPACT P2Y12 expected values are typically 0-15 ohms*min, with percent inhibition of IMPACT ADP > 70%. However, each laboratory should establish the normal range.

Section 15: Interpretation of results
For Research Use Only. Substances such as caffeine, tobacco, alcohol and vitamin C may also affect the result. [6,8]

Section 16: Performance Characteristics:

i. Analytical Performance Characteristics
From testing at Hart site, aggregation response for IMPACT P2Y12 testing were as follows over 20 tests:
Citrate Sample:
AUC: 0 – 15 ohms*min.
Max Aggregation: 0 – 10 ohms.
Max Slope: 0 – 10 ohms/min.
Hirudin Sample:
AUC: 0 – 15 ohms*min.
Max Aggregation: 0– 10 ohms.
Max Slope 0 – 10 ohms/min.

ii. Performance Characteristics
For Research Use Only. IMPACT P2Y12 – For Research Use Only, may be used in platelet studies alongside IMPACT ADP – For Research Use Only, to inhibit the P2Y12 receptor of platelets.[6,8,10]

iii. Measuring Intervals
IMPACT P2Y12 – For Research Use Only, when used according to semi-automated test protocol of the IMPACT analyser, the final concentration is 0.625µg/ml.

Section 17: Biological reference intervals
For Research Use Only. Each laboratory should define its acceptable normal aggregation ranges with use of normal healthy donors and verify the expected performance characteristics.

Section 18: Limitations of the examination procedure
For Research Use Only.
For use by trained professionals only.
Impaired platelet function has been reported after the ingestion of several drugs and herbal medicines. Aggregation can be abnormal in thrombocytopenia samples.

Section 19: Literature References:

1. Ranucci, Marco; Simioni, Paolo (20 January 2016). Point-of-Care Tests for Severe Hemorrhage: A Manual for Diagnosis and Treatment. Springer. pp. 40–42. ISBN 978-3-319-24795-3
2. Marcucci, Carlo; Schoettker, Patrick (17 September 2014). Perioperative Hemostasis: Coagulation for Anesthesiologists. Springer. pp. 54–56. ISBN 978-3-642-55004-1.3.
3. Dacie & Lewis, 'Practical Haematology', Lewis, S.M., Bain, B.J. and Bates, I. (Editors); 9th Edition, Elsevier Science Ltd., 2002, pages 380-385.
4. 'WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy', World Health Organisation, 2010, ISBN 978 92 4 159922
5. CLSI. Platelet Function Testing by Aggregometry; Approved Guideline. CLSI document H58-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
6. BSCH Guideline Harrison P. Et al. Guidelines for the laboratory investigation of heritable disorders of platelet function.
7. Kaiser AF, Neubauer H, Franken CC, et al. Which is the best anticoagulant for whole blood aggregometry platelet function testing? Comparison of six anticoagulants and diverse storage conditions. Platelets. 2011 Oct 14.
8. J.J.J. Van Giezen, L. Nilsson, P. Berntsson et al. Ticagrelor binds to human P2Y12 independently from ADP but antagonizes ADP-induced receptor signaling and platelet aggregation. Journal of Thrombosis and Haemostasis. 2009; 7(9):1556-1565.
9. Tóth O., Calatzis A., Penz S., Losonczy H, Siess W.: Multiple electrode aggregometry: A new device to measure platelet aggregation in whole blood; Thromb Haemost 2006; 96: 781-8
10. Aradi D, Komócsi A, Price MJ, et al. Efficacy and safety of intensified antiplatelet therapy on the basis of platelet reactivity testing in patients after percutaneous coronary intervention: systematic review and meta-analysis. Int J Cardiol. 2013;167(5):2140–8.