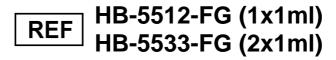


# **TRAP-6 Reagent**



## Guide to Symbols



Consult accompanying documents Voir documents cijoints Siehe beigefügte, Dokumente Véanse los documentos djuntos Vedi documenti allegati



For *in vitro* diagnostic use Pour usage diagnostique *in-vitro* in-vitro diagnostikum Para uso diagnóstico *in-vitro* Per uso diagnóstico *in-vitro* 



Store at 2-8°C Conserver à 2-8°C Lagern bei 2-8°C Conservar a 2-8°C Conservare a 2-8°C



Use By A utiliser avant le Verw. Bis: Utilizar antes de Usar entro



Lot Lot Ch.-B.: Lote



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.com, e-mail: info@hartbio.com





# TRAP-6 Reagent

### INTENDED PURPOSE



Hart Biologicals Thrombin Receptor Activator Peptide -6 (TRAP-6) Reagent is used to diagnose platelet dysfunction, or normal platelet activity in human platelet rich plasma or whole blood.

### SIIMMADV

SUMMARY
TRAP-6 is a synthetic hexapeptide that activates the thrombin receptor independent of receptor cleavage. It corresponds to amino acids 42 to 47 of the thrombin receptor and mimics the effects of thrombin. In Thrombin is a very potent platelet activator. Its action is not blocked by anti-platelet drugs such as Aspirin or Clopidogrel. TRAP-6 can be used as an alternative to Thrombin as a platelet activator as it is easily standardized and more stable than thrombin. TRAP-6 can be used to detect the action of Gpllb/Illa antagonists such as Reopro, Aggrastat or Integrillin.<sup>5</sup>.

The platelet aggregation test measures the rate and degree to which dispersed platelets in a sample of platelet rich plasma (PRP) or anticoagulated whole blood forms clumps (aggregates) after the addition of a substance that normally stimulates platelet aggregation (agonist). In optical aggregometry, the clumping of the platelets causes the platelet rich plasma to become less turbid. This is measured on a platelet aggregometer, which plots the rate and maximum extent of the aggregation reaction. In whole blood aggregometry, 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.

### WARNINGS AND PRECAUTIONS

For in-vitro diagnostic use only.

Do not pipette by mouth. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.

Wear disposable gloves when handling specimens and kit reagents, and wash hands thoroughly afterwards.

### TRAP-6 Reagent

Interviseage in Ingredients: The reagent contains a lyophilised preparation of 1mM TRAP-6 with added buffer and stabilisers.

Preparation for use: Reconstitute each vial of TRAP-6 reagent with 1.0ml of distilled or deionised water as indicated on the vial label. Allow to stand for 10 Storage and stability: The lyophilised product should be stored at 2...8°C and is stable until the expiry date printed on the vial label. After reconstitution, the product is stable for 8 hours at room temperature (20...25°C), 2 weeks at 2.8°C or 4 weeks at -20°C.

### MATERIALS REQUIRED BUT NOT PROVIDED

Platelet aggregometry system – the Hart Biologicals TRAP-6 Reagent will perform satisfactorily when used on any aggregometer system. Follow the manufacturer's instructions for the operation of the aggregometer in use.

Purified water.

Pipette for 1.0ml

SAMPLE COLLECTION AND PREPARATION<sup>4</sup>
Preparation of Platelet-Rich and Platelet- Poor Plasma for Optical Aggregation
Blood for platelet aggregation testing should be collected in to plastic syringes and transferred to plastic tubes, or collected in siliconised glass evacuated blood

- collection tubes.

  Blood (9 parts) should be mixed with 0.11M or 0.13M sodium citrate anticoagulant (1 part), Invert gently to mix. Do not shake.

  Prepare platelet rich plasma by centrifuging the anticoagulated blood at 150-200 x g for 10-15 minutes at room temperature.

  Remove the platelet rich plasma with a plastic transfer pipette and place in a plastic container (with cap) labelled 'PRP'. Cap the container and keep at room temperature.

  Prepare platelet poor plasma by centrifuging the remaining blood specimen at 2000 x g for 20 minutes.

  Remove the platelet poor plasma with a plastic transfer pipette and place in a plastic container (with cap) labelled 'PPP'. Cap the container and keep at room temperature.

  - keep at room temperature
- Adjust the platelet concentration in the PRP to 200-300x10<sup>9</sup>/L using PPP, cap and allow to stand at room temperature for 30 minutes prior to testing.

  Testing should be completed within 3 hours of blood collection.

  Whole Blood Aggregation Samples

  Refer to the aggregometer manufacturer recommendations for the preparation of samples for whole blood aggregometry.

### TEST PROCEDURE

- Optical Aggregometry
  Set the 0% and 100% aggregation levels on the aggregometer using platelet poor plasma and platelet rich plasma according to the manufacturer's instructions.
- institutions.

  Pipetite the required volume of platelet rich plasma in to an aggregation cuvette and add a stir bar.

  Pre-warm to 37°C for 120 seconds.

  Add the required volume of TRAP-6 reagent directly in to the cuvette. Do not allow reagent to run down the wall of the cuvette.
- Allow the aggregation pattern to form for a minimum of 5 minutes.
- 5.

### B) Whole Blood Aggregometry

Refer to the manufacturer's instructions for the correct performance of the test.

### QUALITY CONTROL

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 ingested aspirin or aspirin containing compounds in the preceding 10 days and should not be on any other form of anti-platelet medication.

### EXPECTED VALUES 5,6

-6 can be used as part of a series of aggregation tests to monitor anti-platelet drugs

Aggregation Response Aspirin Clopidogrel Aggrastat Reduced or absent Reduced or absent

### **FURTHER TESTING**

If the test results are abnormal, the test should be repeated on a separate occasion. If the results are consistently abnormal, and the patient is not taking any medication known to interfere with platelet function, additional tests should be considered.

Integrillin

In optical aggregometry, the presence of red blood cells in the PRP will cause the total observed aggregation to be reduced. The presence of platelets in the PPP will cause the total observed aggregation to appear increased. Spurious results can be observed when the total platelet count of the PRP is less than 75 x 10°/L. PRP tested less than 30 minutes after preparation may exhibit abnormal aggregation profiles.

### graphy

Reduced or absent

- praphy
  Coughlin, S.R. Protease-activated receptors and platelet function', Thromb. Haemost., 1999; 82: 353
  Coughlin, S.R. Protease-activated receptors in vascular biology, Thromb. Haemost., 2001; 86: 298
  Vassallo, R.R. N.Ir., Kieber-Emmons, T., Cichowski, K. et al. Structure-function relationships in the activation of platelet thrombin receptors by receptor-derived peptides' J. Biol. Chem., 1992; 267; 6081
  Andersen, H., Greenberg, D.L., Fujikawa, K. et al. Protease-activated receptor 1 is the primary mediator of thrombin-stimulated platelet procoagulant activity. Proc. Natl. Acad. Sci. USA, 1996; 96: 11189
  Dynabyte Medical, Tests for Multiplate analysis. http://www.multiplate.net/GB/MultiplateTests.html
  Dacie & Lewis, 'Practical Haematology', Lewis, S.M., Bain, B.J. and Bates, I. (Editors); 9<sup>th</sup> Edition, Elsevier Science Ltd., 2002, pages 383-385.
  Dacie & Lewis, 'Practical Haematology', Lewis, S.M., Bain, B.J. and Bates, I. (Editors); 9<sup>th</sup> Edition, Elsevier Science Ltd., 2002, pages 384-385. 3.
- 4.