BIO/DATA CORPORATION

TRAP-6 amide Kit

REF 106956

RESEARCH USE ONLY

Product Description

NON STERILE

TRAP – 6 amide reagent is a lyophilized, synthetic hexapeptide fragment of the thrombin receptor. (SFLLRN-NH). It is a PAR -1 antagonist. TRAP – 6 reagent irreversibly induces platelets to aggregate, undergo structural changes, and to degranulate.[1-4]

Test Principle

TRAP – 6 reagent induces platelet aggregation.

Light Transmission Aggregometry (LTA) is the reference method for the measurement of platelet function.[5] The dynamic range is established by the difference in light transmission between Platelet Poor Plasma (PPP) which sets the 0% baseline and Platelet Rich Plasma (PRP) which sets the 100% limit.

When added to normal Platelet Rich Plasma, or certain other platelet preparations, TRAP - 6 reagent elicits a strong, monophasic response in a concentration dependent manner. [5]

Materials Provided

A single vial containing 5.0 mg of lyophilized TRAP – 6 reagent. Its molecular weight is 747.90.

Materials Required but not Provided

1. Platelet Aggregometer
2. Preservative Free Physiologic Saline (0.85 or 0.90%)
3. Pipettes and tips
4. Sample tubes and caps
5. Siliconized Aggregometer cuvettes
6. Plastic coated micro stir bars

Follow the aggregometer manufacturer's INSTRUCTIONS for USE and sample size and agonist volume requirements.

FOR PROFESSIONAL LABORATORY USE ONLY

Reagent Storage

TRAP 6 Reagent may be transported at ambient temperature but should not be exposed to prolonged periods at high temperatures. Once received, unreconstituted TRAP – 6 reagent should be stored at -20°C or colder temperatures.

Reconstituted TRAP – 6 reagent may be stored for extended periods at -80°C.

Reconstitution

TRAP – 6 reagent must be warmed at 37°C, then equilibrated to room temperature prior to use.

Tap the top of the vial gently to minimize any potential loss of material.

DO NOT reconstitute TRAP – 6 reagent in its original vial.

Reconstitute TRAP – 6 reagent with preservative free, physiologic saline (0.85 or 0.9%). Select the desired amount of TRAP – 6 reagent and saline from one of the tables below or follow laboratory protocol.

Reconstituted TRAP – 6 reagent should be stored at 2 – 8 °C.

Stability

2 – 8°C: two weeks (reconstituted reagent)
-20°C: two years (lyophilized or aliquots)
-80°C: five years (lyophilized or aliquots)

Dilution Fomula

Concentration 1	Volume 1	Concentration 2	Volume 2
100 micromolar X	1 milliliter =	10 micromolar X	10 millileter
C1	V1	C2	V2

Use one of the online versions of this formula for calculating

Table 1: Preparation of Micro-molar (µM) Concentrations

Milligrams of TRAP-6 Reagent	Milliliters of Diluent	TRAP-6 Working Concentrations
1.0	8.9	150 µM
1.0	13.4	100 µM
2.0	17.8	150 µM
2.0	26.7	100 µM
2.5	22.3	150 µM
2.5	33.4	100 µM

Table 2: Preparation of Milli-molar (mM) Concentrations

Milligrams of TRAP-6 Reagent	Milliliters of Diluent	TRAP-6 Working Concentrations
5.0	1.0	6.7 mM
5.0	2.0	3.3 mM
5.0	3.0	2.2 mM
5.0	4.0	1.7 mM
5.0	5.0	1.3 mM

Table 3: Preparing Further Micro (µ) Molar (M) Dilutions of TRAP-6

Milligrams of 100 µM TRAP-6	Milliliters of Diluent	Working Concentration (µM) TRAP-6
1	2.0	50.0
2	2.5	40.0
3	1.67	30.0
4	1.25	20.0
5	2.5	10.0

Reagent Disposal

Unused TRAP -6 reagent must be disposed of as a hazardous material in accordance with local regulations and laboratory policy.

Test Procedure: Light Transmission Aggregometry (LTA)

1.Place the appropriate number of test cuvettes into the incubation wells.
2. Add a new, plastic coated stir bar to each cuvette.
3. Prepare the PPP blank by pipetting 0.250 µl of PPP into a cuvette.
DO NOT PLACE A STIR BAR IN THE BLANK TUBE
4. Pipette 0.225 µL of PRP (patient sample) into each test cuvette containing a stir bar.

5. Place the PRP sample tubes in the incubation block
    a. Select the timer button for the test channel, and a countdown will begin.
    b. Incubate the PRP test samples for a pre-set incubation period and temperature (37.0°C)
6. Set the 100% baseline by placing the blank into the test well.
    a. Press the Blank Button
    b. Remove the Blank from the test well
7. Place the PRP sample cuvette into the test well
    a. Press the Start Button.
8. Add 0.25 µL of the agonist/reagent into the PRP using the proper pipette and tip to assure the agonist/reagent is directed into the center of the cuvette and not allowed to run down the side of the cuvette.
9. Select inject
10. The test will run for the pre-set test-time.(~ 6 minutes)
11. An alarm will sound when testing in all channels is completed.

References

1. Scarborough, RM et. al. J. Bio Chem. 267, 13146-1992.

2. Reese, MJ. et. al. Near-Patient Platelet Function Testing in Patients Undergoing Coronary Artery Surgery: A Pilot Study, Anesthesia, 2011.66, p 97-103. doi 10.1111/j.1365-2044.06608.x.

3. Horpus, DL et.al. Creatine Kinase Inhibits ADP Included Platelet Aggregation. Scientific Reports. 2014; 4: 6551. Published 10/9/14. doi. 10.1038/srep06551

4. Dobrovolskaia, M. A and McNeil, SE. Frontiers in Nanobiomedical Research, Vol 1. Handbook of Immunologic Properties of Engineered Nanomaterials (5.1.2) World Scientific. Singapore, Hackensack, London. 2013. ISBN:978-981-4699-16-7

5. Clinical and Laboratory Standards Institute (CLSI). Platelet Function Testing by Aggregometry. Approved Guideline. CLSI document H 58A. (SBN 1-56238-683-2). CLSI, 950 West Valley Road, Suite 2500, Wayne, PA.

BIO/DATA CORPORATION

155 Gibraltar Road, Horsham, PA 19044-2303 U.S.A.
(800) 257-3282 U.S.A. (215) 441-4000 Worldwide
(215) 443-8820 Fax Worldwide
E-mail: customer.service@biodatacorp.com
Internet: www.biodatacorp.com
An ISO 13485:2016 Registered Company