AMERICAN BIOCHEMICAL & PHARMACEUTICALS LTD.

REF ABP-ADP-1
3 X 0.5mL, Lyophilized
adenosine-5-diphosphate

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PRODUCT DESCRIPTION
ADP Reagent is a lyophilized preparation of adenosine – 5 – diphosphate.

INTENDED USE
ADP is intended for use in routine platelet aggregation studies for the evaluation of inherited or acquired platelet dysfunctions, platelet activation and the effects of anti-platelet therapy.

TEST PRINCIPLES
In Light Transmission Aggregometry (LTA), when ADP is added to Platelet Rich Plasma, platelets activate, change shape and aggregate. This is primary aggregation and is reversible (disaggregation). 1 This results in an increase in the amount of light transmitted through the sample (% Aggregation). Normal platelets will further respond by releasing endogenous ADP from dense granules, causing a second, irreversible wave of aggregation.[1,2,3]

MATERIALS PROVIDED
ADP Reagent, 3x0.5mL

MATERIALS REQUIRED BUT NOT PROVIDED
1. Platelet Aggregometer
2. Purified Water (distilled, deionized or reagent grade) (pH 5.3 - 7.2)
3. Electronic Pipettors
4. Pipette tips
5. Non-platelet activating plastic sample tubes and caps
6. Siliconized Aggregometer cuvettes
7. Plastic coated micro stir bars

INSTRUMENTATION
ADP Reagent will perform as described when used in accordance with these instructions on most Light Transmission Aggregometers.

Laboratories must follow the Instructions for Use for the aggregometer to be used to perform the test.

RECONSTITUTION and REAGENT STORAGE
1. Bring reagents to room temperature (15 – 28 °C) prior to reconstitution
2. Reconstitute each vial of ADP Reagent with 0.5 mL of purified water
3. Refrigerated (reconstituted) reagent must be brought to room temperature prior to use
4. Reconstituted Reagent is stable for 30 days when stored at 2 – 8 °C in its original, tightly sealed container.

REAGENT DISPOSAL
ADP is an IVD product and not intended for injection or other use.

Biological products must be handled with all required precautions and should be considered potentially infectious.

Unused or expired ADP reagent must be disposed of as a hazardous waste in accordance with local regulations and laboratory policy.

PROFESSIONAL LABORATORY USE ONLY

PERFORMANCE CHARACTERISTICS
ADP Reagent will perform as indicated prior to its expiration date when procedural and storage directions are followed.

BIPHASIC AGGREGATION
To elicit a biphasic response to ADP, the Platelet Rich Plasma (PRP) may have to be challenged with a number of ADP concentrations. These final concentrations can range from 0.2 µM to 20.0 µM. Table 1 below shows how to prepare these dilutions from the reconstituted vial of ADP (working concentration 200 µM).

Always use preservative free, physiologic saline (0.8, 0..85, or 0.9%) to dilute the ADP.

TABLE 1: RECOMMENDED ADP DILUTIONS FOR ELICITING A BIPHASIC RESPONSE.[4]

ADP STOCK SOLUTION (µL)	PHYSIOLOGIC SALINE (µL)	WORKING CONCENTRATION (µM)	FINAL CONCENTRATION (µM)
---	---	200.0	20.0
125.0	125.0	100.0	10.0
62.0	188.0	50.0**	5.0
50.0	200.0	40.0	4.0
38.0	212.0	30.0	3.0
25.0	225.0	20.0****	2.0
12.0	238.0	10.0	1.0
25.0 OF**	225.0	5.0	0.5
25.0 OF****	225.0	2.0	0.2

EXPECTED VALUES* (test time 6 minutes)

ADP	WORKING CONCENTRATION	FINAL CONCENTRATION
	200.0 µM	20.0 µM

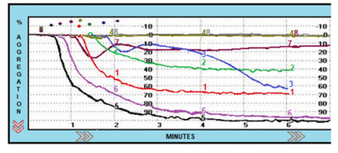
LAG PHASE	PRIMARY SLOPE	FINAL AGGREGATION @ 6 MINUTES (%)	BIPHASIC AGGREGATION	AUC@ 6 MINUTES
≤ 10	38-70	62 - 101	Concentration Dependent	

NOTE: May show shape change

RESULTS
Typical ADP aggregation patterns.

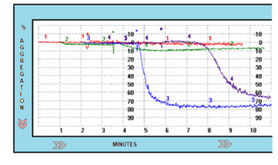
ADP, at final concentration of 20µM, will induce a large single wave of aggregation in normal platelet rich plasma. At a final concentration (in test) of 2µM to 10 µM, two waves of aggregation may be observed. The primary wave is the response to the exogenous ADP (reagent). The secondary wave is due to the release of endogenous ADP from the non-metabolic pool of nucleotides (storage pool) contained within the platelets.[5]

ADP CONCENTRATION/RESPONSE IN HEALTHY DONORS



CHANNEL	ADP CONCENTRATION	CHANNEL	ADP CONCENTRATION
1	10.0 µM	5	10.0 µM
2	5.0 µM	6	5.0 µM
3	2.0 µM	7	2.0 µM
4	1.0 µM	8	1.0 UM

EFFECT OF 81 mg ASA on COMMON AGONISTS



CHANNEL	AGONIST
1	SALINE (SPONTANEOUS AGGREGATION
2	ARACHIDONIC ACID
3	ADP
4	COLLAGEN

LINEARITY
Platelet Aggregation is a non-linear test system. Platelet Aggregation can be induced by a variety of agents. It is a biological reaction. The underlying reaction, test conditions/instrumentation type, agonist and agonist concentration, among other factors contribute to reaction response. Platelet Aggregation is not a quantitative test. It measures the rate and extent of a response to the agonist in a concentration dependent manner.
The following parameters are reported for agonist induced platelet aggregation: Primary Aggregation, Primary Slope, Secondary Aggregation, Secondary Slope (biphasic response), Lag Phase, Disaggregation, AUC @ 6 minutes, Maximum aggregation, and Final aggregation.

ACCURACY, PRECISION AND REPRODUCIBILITY

Accuracy

Accuracy is a relative parameter in Light Transmission Aggregometry (LTA). It depends on the test system.

Precision and Reproducibility

The nature and limitations of LTA make it difficult to provide the usual precision or reproducibility ranges for the test. Consensus reports refer to the following ranges and experts recommend that each laboratory establish its own limits for test acceptability.[6,7,8]

Test to Test Reproducibility:	better than ± 7.5%
Instrument to Instrument Reproducibility:	better than ± 15%
Reagent Lot to Lot Variation:	better than ± 10.5%

QUALITY CONTROL

Westgard's Rules for low volume tests should be followed where practical.[12]

The use of a known donor is recommended for control and interpretation purposes. Low volume laboratories should include a known donor on each day of tests. Higher volume laboratories should choose the frequency of known donor use that is appropriate for the test volume and operator experience. Limited Proficiency Test Programs are available from The College of American Pathologists and NASCOLA. Contact information is provided at the end of the reference section.

LIMITATIONS

A detailed patient history is required for accurate test interpretation. Patients should be questioned about the recent ingestion of any medication because a number of prescription and nonprescription drugs may interfere with platelet aggregation. Substances such as caffeine, tobacco, herbal extracts (or supplements) and alcohol may affect results.

TEST PREPARATIONS

PATIENT PREPARATION[6,8,9,10]

1. Prior to being tested, Clinical, medication, family and social histories are required.
2. Patients should refrain from taking Aspirin or other anti-platelet medications for 7-10 days, or as directed by their physician.
3. Patients should avoid supplements, herbal preparations, energy drinks or other products known to affect platelet function.
4. Patients should avoid fatty meals and food products prior to specimen collection.

SPECIMEN COLLECTION[6,7,11,12]

Refer to the current CLSI Approved Guidelines H 58 -A: Platelet Function Testing by Aggregometry for detailed specimen collection and sample preparation instructions and related references.

EVACUATED SPECIMEN COLLECTION TUBE TECHNIQUE (PREFERRED)

1. Use a 21 or 23 gage winged needle set for specimen collection
2. Remove the tourniquet as soon as blood starts to flow
3. Collect the blood specimen in 2.7µL plastic evacuated specimen collection tubes containing 0.105/0.11 M (2.3%) buffered sodium citrate anticoagulant.
4. Gently invert each tube 4 -5 times to assure complete mixing.
5. Maintain specimens at room temperature without removing the caps.
6. Observe Standard Precautions through out the specimen collection process and follow appropriate laboratory policies for post phlebotomy patient care and disposal of sharps and supplies.

1. Evacuated specimen collection tubes with light blue tops may contain 3.2% or 3.8% sodium citrate. Check the label for the proper concentration.[6,13,14,15]
2. Underfilled tubes should be rejected
3. Blood collection should be performed with care to avoid patient anxiety, stasis, hemolysis and contamination by tissue fluid, or any exposure to glass.
4. Make sure the winged needle set is intended for phlebotomy use.
5. Each of the following can cause test results to be inaccurate
   a. Visible RBC contamination
   b. Hemolysis
   c. Icterus
   d. Lipemia
   e. Clots

These are unacceptable specimens and should be rejected.

6. Test results may also be affected if the patient has thrombocytopenia (thresholds are agonist and analyzer dependent) or hypofibrinogenemia. Follow laboratory policies when such specimens have been collected.
7. If the patient's hematocrit is less than 30% or greater than 55%, the blood to anticoagulant ratio must be adjusted. (see H58 -A for instructions)
8. Specimens must be tested within four hours of collection.

SAMPLE (PRP & PPP) PREPARATION[6,10,15,16]

PREPARATION OF PLATELET RICH PLASMA (PRP) & PLATELET POOR PLASMA (PPP) TEST SAMPLES

Check the RCF Nomogram in the centrifuge manual to confirm the proper settings.

1.Prepare Platelet Rich Plasma test samples first.
2.Centrifuge the unopened specimen collection tubes at 150 x g for 10 minutes at room temperature.
3. Do not engage the centrifuge's brake.
4. Carefully remove the tubes from the centrifuge. Examine the plasma layer for the presence of Red Blood Cells (RBCs)
   a. If there are RBCs present, re-centrifuge for an additional five minutes at 150 x g.
5. Using a plastic transfer pipette, carefully remove the PRP layer without disturbing the buffy coat and transfer the PRP to labeled plastic sample tubes and cap the tubes. Maintain the PRP at room temperature.
6. To prepare the PPP, recap the specimen collection tubes and re-insert them into the centrifuge. Centrifuge those specimens at 1500 x g for 20 minutes.
7. Check for hemolysis.
   a. If the PPP is hemolyzed, it is unacceptable for use as a blank.
8. Carefully transfer the PPP to pre-labeled plastic tubes and cap them. Maintain them at room temperature.[6,9]

1. PRP should have nominal platelet counts greater than 200,000/cumm
2. PPP must have a platelet count less than 10,000/cumm
3. Platelet counts on PRP and PPP can not be performed using automated hematology analyzers. Those analyzers were neither designed or intended for counting these samples. It is best to count PRP and PPP, if necessary using a hemocytometer.
4. PRP platelet counts should not be adjusted using PPP.
5. PRP has a maximum useful life of four hours from the time of collection.

GENERIC LTA TEST PROCEDURES

1. Place the appropriate number of test cuvettes in to 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 in to 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°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.
11. An alarm will sound when testing in all channels is completed.

ADP RESPONSES

CONDITION	ADP RESPONSE
Glanzmann's Thrombasthenia	Absent
Bernard Soulier Syndrome	Normal
von Willebrand Disease	Normal
vWD Type 2b	Normal
Storage Pool Disease	Decreased or Abnormal
Myeloproliferative Disease	Normal or Absent
Quebec Platelet Disorder	Absent
Hyperaggregation	Increased at low conc.
Aspirin	Normal or Decreased
Thienopyridines	Aggregation Inhibited

FURTHER TESTING:
If the test results are abnormal when properly interpreted:
1. Review clinical history
2. Review the patient's medication record
3. Recheck the patient's social history for use of aspirin containing compounds, supplement use and herbal/spice use.

WARRANTY

This product is warranted to perform to these specifications when used in accordance with labeling. American Biochemical and Pharmaceuticals Ltd. disclaims any implied warranty of merchantability and fitness for any other purpose and in no event shall American Biochemical and Pharmaceuticals Ltd. be liable for any consequential damages arising out of the aforesaid warranty.

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Printed in U.S.A. P/N 107498 Rev.-