BIO/DATA CORPORATION

ADP
(ADENOSINE-5'-DIPHOSPHATE)

PRODUCT DESCRIPTION
ADP is a lyophilized preparation of adenosine-5'-diphosphate. The working concentration of the reconstituted reagent is 200 µM, see Table 1

INTENDED USE
ADP reagent is for routine use in eliciting a concentration dependent activation or aggregation response in a Platelet Rich Plasma sample.

PRINCIPLE
When added to platelet rich plasma, ADP stimulates platelets to change their shape and aggregate. Aggregation induced by exogenous ADP is referred to as primary aggregation and is reversible. Normal platelets will further respond by releasing endogenous ADP from their granules. Release of endogenous ADP results in a secondary wave of aggregation which is irreversible.[8,10,11]

PRECAUTIONS
ADP is for Professional Laboratory Use Only. ADP is for *IN-VITRO* DIAGNOSTIC REAGENT USE ONLY AND NOT FOR INJECTION OR INGESTION.

MATERIALS PROVIDED
ADP, 3 x 0.5mL. Store at 2° to 8° C prior to reconstitution.

MATERIALS REQUIRED BUT NOT PROVIDED
1. Platelet Aggregometer
2. Purified water (distilled, deionized or reagent grade), pH 5.3 - 7.2
3. Pipettors
4. Disposable Stir bars
5. Aggregometer cuvettes

INSTRUMENTATION
ADP will perform as described when used on most optical platelet aggregometers[1]. Follow the manufacturer's instructions for operating the aggregometer in use.

SPECIMEN COLLECTION AND PREPARATION OF TEST SAMPLE

Refer to the current NCCLS Approved Guideline H21 A2 for detailed specimen collection and sample preparation instructions.[6]

1. **PATIENT PREPARATION:**
   Patients should refrain from taking aspirin or medications containing aspirin, other medications and dietary supplements known to affect platelet function for 7 - 10 days prior to specimen collection. Patients should fast and avoid fatty foods and dairy products for 12 hours prior to specimen collection.[6]

2. **SPECIMEN COLLECTION:**
   Blood collection should be performed with care to avoid stasis, hemolysis, contamination by tissue fluids, or exposure to glass. Keep specimens at room temperature.[8]

   Each of the following can cause test results to be inaccurate; and affected specimens should be rejected: hemolysis, RBC contamination, lipemia, chylous, icterus, thrombocytopenia (<75,000/mm^3) clots in specimen, and hypofibrinogenemia. Reuse of disposable items may result in inaccurate test results.

   Observe standard precautions throughout the specimen collection, sample preparation and analytical processes.[2,3] Dispose of sharps and biological waste in accordance with laboratory policy.

Evacuated Collection Tube Technique
1. Use a butterfly needle for the venipuncture.
2. Draw blood using (plastic) tubes containing 0.11M Sodium Citrate anticoagulant.
3. Gently invert 4-5 times to mix.

NOTE: When using plastic vacuum collection tubes, make sure the 3.2% sodium citrate anticoagulant is 0.11M by checking the label. Colored tops do not vary with differing citrate concentrations. Follow the manufacturer's instructions for specimen collection.

Syringe Technique
a. Use a butterfly needle for the venipuncture.
b. Draw 9.0mL of blood into a plastic syringe. Avoid excess suction.
c. Remove the needle from the syringe and immediately and gently dispense the blood into a plastic [polypropylene][4] tube containing 1.0mL of 0.11M Sodium Citrate anti-coagulant. The ratio of blood to anti-coagulant must be 9 parts of blood to 1 part anti-coagulant.[5]
d. Cover and invert 4-5 times gently to mix.
e. Maintain in a vertical position and at room temperature (15° to 28°C).

NOTE: When the patient's hematocrit is < 30% or > 55%, the blood to anticoagulant volumes must be adjusted.[4]

PREPARATION OF PLATELET RICH PLASMA (PRP) AND PLATELET POOR PLASMA (PPP)
1. Prepare platelet rich plasma by centrifuging the anti-coagulated blood at 150 X g for 10 minutes at room temperature(15°to28°C).
2. Examine the plasma layer for red cells. If red cells are present, re-centrifuge at 150 X g for an additional 5 minutes.
3. Using a plastic transfer pipette, observe and carefully remove the platelet layer without disturbing the buffy coat or red cells, and transfer to a container labeled (PRP). Cap the container and allow it to stand at room temperature.
4. Prepare the platelet poor plasma by centrifuging the remaining blood specimen at 2500 x g for 20 minutes. Examine the platelet poor plasma for hemolysis, then transfer it to a plastic tube labeled PPP.

RECONSTITUTION
NOTE: Reagents must be at room temperature (15° to 28°C) prior to reconstitution. Stored reagent must be brought to room temperature prior to use.

Reconstitute a vial of ADP with 0.5mL purified water.

REAGENT STORAGE
The reconstituted ADP is stable for 30 days when stored at 2° - 8°C in its original tightly sealed container.

TEST PROCEDURE
Testing must be completed within 4 hours of specimen collection.[8]
1. Place the appropriate number of cuvettes required for testing into the incubation wells
   a. add a stir bar in each cuvette
2. Prepare a blank by pipetting 0.250mL platelet poor plasma (PPP) into a cuvette.
   a. do not add a stir bar.
3. Pipette 0.225mL of the sample platelet rich plasma (PRP) into a pre-warmed cuvette for each patient to be tested.
4. Place the cuvette of sample PRP plasma into the incubation well.
   a. select the timer button and the count down will begin
   b. incubate the sample at 37°C for the preset time.
5. Set the 100% baseline by placing the blank into the test well.
   a. select the blank button
6. Place the cuvette of sample PRP plasma into the test well.
   a. select start
7. Add 0.025mL reagent directly into the platelet rich plasma
   a. select inject
      I. do not allow reagent to run down the wall of the tube.
8. The test will run for pre-set time (6min).
   a. other manufacturer's test procedures may specify different times.

BIPHASIC AGGREGATION
To demonstrate 2 distinct waves, or "biphasic" ADP aggregation, the platelet rich plasma may be tested with various dilutions of the reagent.[10]

Prepare the diluted concentrations of ADP as follows:
Always use 0.85% or 0.90% saline for dilutions.

Table 1

ADP	Saline	Working Concentration	Final Concentration
------	------	200 µM	20 µM
125 µL	125 µL	100 µM	10 µM
62 µL	188 µL	50 µM*	5 µM
50 µL	200 µL	40 µM	4 µM
38 µL	212 µL	30 µM	3 µM
25 µL	225 µL	20 µM**	2 µM
12 µL	238 µL	10 µM	1 µM
25 µL of *	225 µL	5 µM	0.5 µM
25 µL of **	225 µL	2 µM	0.2 µM

QUALITY CONTROL
Laboratories should follow generally accepted quality control practices when proficiency testing is not available.

To assure proper instrument operation and reagent performance, a control specimen should be evaluated each day that tests are performed. The control specimen should be prepared in the same manner as the test specimen. For qualitative platelet aggregation studies, the control should consist of fresh platelet rich plasma collected from a (specified and qualified) normal donor who has not ingested aspirin containing compounds within 10 days of testing and has a history of normal platelet function.[12,13,14]

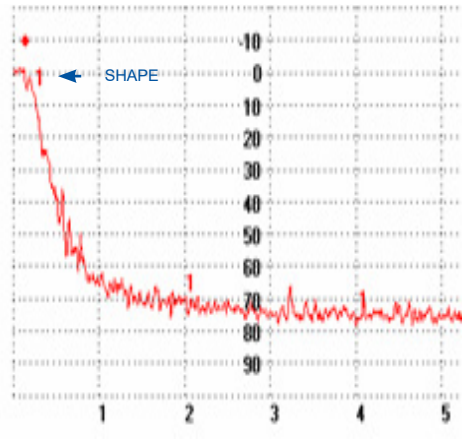

Figure 1 Normal Aggregation
(Final Concentration 20 µM), See Table 1.

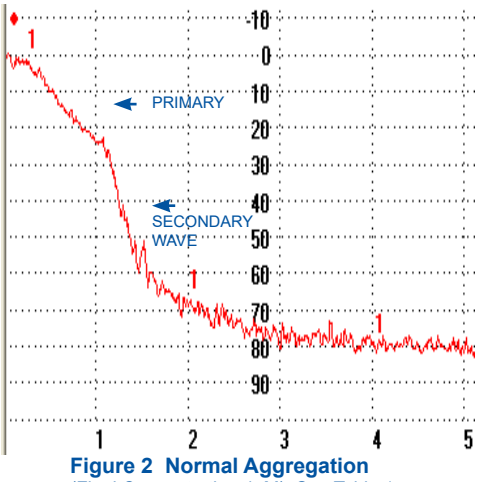

Figure 2 Normal Aggregation
(Final Concentration 4µM), See Table 1.

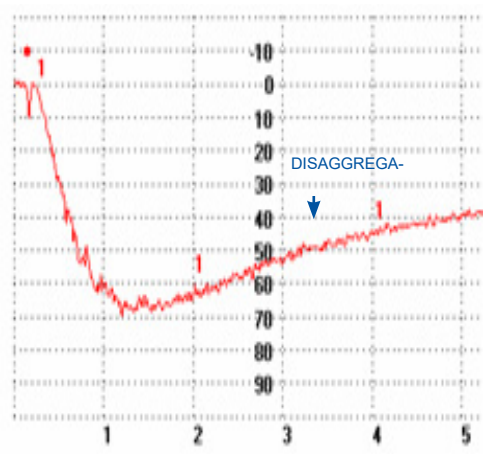

Figure 3 Abnormal Aggregation
(Final Concentration 20µM), See Table 1.

LEGEND: Results of ADP-induced platelet aggregation on normal and abnormal platelet rich plasma.

Spike mark indicates addition of reagent. Concentration dependent disaggregation may be observed in some normal PRP. This phenomenon is illustrated in Figure 3.

RESULTS

Typical ADP aggregation patterns are illustrated in Figures 1 - 3.

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 (See Figure 2). 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.[9]

EXPECTED VALUES

Expected ranges for each reagent at various concentrations used to induce platelet aggregation should be established by each laboratory, see Table 2.[4,8,9,10]

TYPICAL PLATELET AGGREGATION RESPONSES FOR NORMAL DONORS @ 250,000 PLATELETS/mm³ [total aggregation at 5 minutes]

Table 2

	ADP	Arachidonic Acid	Collagen [Type I]	Epinephrine
Final Conc.	20 µM	500µg/mL	0.19mg/mL	100 µM
Lag Phase [sec]	<10	<=20	<60	0
Primary Slope	38-70	>20	35-67	7-45
Total Aggregation (%@5min)	62-101	65-90	63-109	54-101
Biphasic Aggregation	concentration dependent	NO	NO	YES
Other	May show Shape changes	All normal Donors may not Conform PLT CT~175k-300k	Do not Dilute	All normal Donors may not Conform

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.[7,8]

PERFORMANCE CHARACTERISTICS

Studies have shown that this product will perform as described prior to its expiration date when procedural and storage directions are followed.

Linearity:

Platelet aggregation induced by common agonists (ADP, Arachidonic Acid, Collagen and Epinephrine) is a nonlinear test system for the following parameters: Lag Phase, Primary Slope, Secondary Slope, biphasic response and disaggregation. The non-linearity is caused by many factors such as the reaction chemistry and instrumentation. Platelet aggregation measures a response rate or activity that is not a quantitative measure of the reactants or their concentration.

ACCURACY, PRECISION AND REPRODUCIBILITY

Accuracy

In platelet aggregation, accuracy is a relative parameter and is dependent on the test system.

Precision and Reproducibility

The limitations of platelet aggregation make it difficult to provide typical precision or reproducibility ranges. However, there is an experienced based consensus for these parameters (see below). Each laboratory must establish its own limits for test acceptability.

Test to Test Reproducibility:	Less than ± 7.5%
Instrument to Instrument Reproducibility:	Less than ± 15%
Reagent Lot to Lot Variation:	Less than ± 10.5%
Laboratory to Laboratory (same test system):	Less than ± 12.5%

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