

PRODUCT DESCRIPTION

ADP is a lyophilized preparation of adenosine-5'-diphosphate. The working concentration of the reconstituted reagent is 2×10^{-4} M, see Table 1.

INTENDED USE

ADP is for use in routine platelet aggregation studies for the evaluation of platelet dysfunction or platelet activation.

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 *IN-VITRO* DIAGNOSTIC 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 (0.5mL, 0.45mL, 0.05mL volumes)
4. Disposable Stir bars
5. Aggregometer cuvettes

INSTRUMENTATION

ADP will perform as described when used on most optical platelet aggregometers¹. 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.⁶

Each of the following can cause test results to be inaccurate; and affected specimens should be rejected: hemolysis, RBC contamination, lipemia, chylous, icterus, thrombocytopenia ($<75,000/\text{mm}^3$) 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.

Syringe Technique (recommended)⁸

- 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] tube containing 1.0mL of 0.11M Sodium Citrate anticoagulant. 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 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

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 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.

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° to 28°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.
5. The platelet count of the PRP should be $250,000 \pm 50,000/\text{mm}^3$. The platelet count may be reduced by using PPP prepared from the sample.

NOTE: If using Arachidonic Acid as an agonist, do not adjust the platelet count.

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 3 hours of specimen collection.⁸

1. Place a stir bar in each cuvette
2. Prepare an aggregometer blank by pipetting 0.5mL platelet poor plasma into a cuvette.
3. Pipette 0.45mL platelet rich plasma into a second cuvette. Incubate at 37° C for 3 minutes.
4. Set, if required, the 0% and 100% baselines according to the manufacturer's instructions for the aggregometer in use.
5. Add 0.05mL ADP directly into the platelet rich plasma. Do not allow reagent to run down the wall of the cuvette. The final concentration of ADP in the platelet rich plasma test mixture is 2×10^{-5} M. See Table 1.
6. Allow the aggregation pattern to generate for 5 minutes.

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:

1. Label 2 test tubes: 4×10^{-5} M and 2×10^{-5} M, see Table 1.
2. Add 0.4mL saline to the tube labeled 4×10^{-5} M, and add 0.2mL saline to the tube labeled 2×10^{-5} M.
3. To make the 4×10^{-5} M: add 0.1mL of the 2×10^{-4} M (from the reconstituted vial) to the tube labeled 4×10^{-5} M. Mix (1 to 5 dilution).
4. To make the 2×10^{-5} M: add 0.2mL of the 4×10^{-5} M (from the 4×10^{-5} M tube) to the tube labeled 2×10^{-5} M. Mix (1 to 2 dilution).
5. Additional dilutions may be prepared using procedures similar to that described in 1-4 above.

Table 1

	Working Concentration	Final Concentration
Reconstituted	2×10^{-4} M	N/A
Normal	2×10^{-4} M	2×10^{-5} M
Biphasic	2×10^{-5} M up to 4×10^{-5} M	2×10^{-6} M up to 4×10^{-6} M

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.

RESULTS

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

ADP, at final concentration of 2×10^{-5} M, will induce a large single wave of aggregation in normal platelet rich plasma. At a final concentration (in test) of 2×10^{-6} M to 4×10^{-6} M, 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

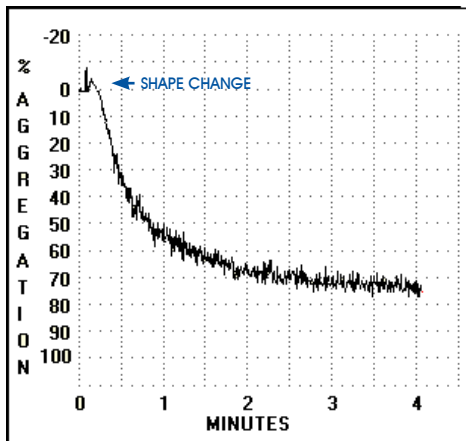


Figure 1 Normal Aggregation
(Final Concentration 2×10^{-5} M), See Table 1.

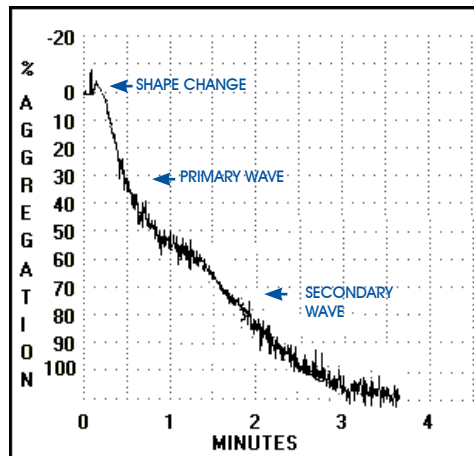


Figure 2 Normal Aggregation
(Final Concentration 2×10^{-6} M), See Table 1.

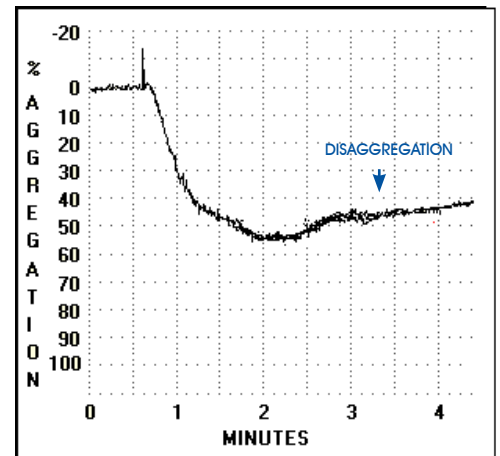


Figure 3 Abnormal Aggregation
(Final Concentration 2×10^{-5} M), See Table 1.

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.

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}

Table 2

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

	ADP	Arachidonic Acid	Collagen [Type I]	Epinephrine
Final Conc.	2.0×10^{-5} M	500µg/mL	0.19mg/mL	1.0×10^{-4} M
Lag Phase [sec]	<10	<=20	<60	0
Primary Slope	38-67	>20	35-67	7-34
Total Aggregation (%@5min)	63-89	65-90	61-99	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:	better than $\pm 7.5\%$
Instrument to Instrument Reproducibility:	better than $\pm 15\%$
Reagent Lot to Lot Variation:	better than $\pm 10.5\%$
Laboratory to Laboratory (same test system):	better than $\pm 12.5\%$

REFERENCES

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PRODUCT AVAILABILITY

PRODUCT	NET CONTENTS	CATALOG NUMBER
ADP	3 x 0.5mL	101312
Arachidonic Acid	3 x 0.5mL	101297
BETA/Pak [®]		
(ADP, Collagen, Ristocetin)	1 x 0.5mL each	101580
Collagen	3 x 0.5mL	101562
Epinephrine	3 x 0.5mL	101311
Lyophilized Platelets	3 x 4 mL	101595
Lyophilized Platelets	1 x 10 mL	101258
PAR/Pak [®] II		
(ADP, Collagen, Epinephrine)	2 x 0.5mL each	101310
Ristocetin		
AggRecetin [®] 1.5mg/mL	15mg	100968
AggRecetin 1.0-1.5mg/mL	15mg	100970
AggRecetin Bulk	100mg	101241
vW Factor Assay [®]	10 Determinations	101246
vW Factor Assay	20 Determinations	103025
vW Abnormal Control Plasma	3 x 0.5mL	101270
vW Normal Reference Plasma	3 x 0.5mL	101269
vW Normal Control Plasma	3 x 0.5mL	106426

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