BIO/DATA CORPORATION

BETA/Pak®

PRODUCT DESCRIPTION
BETA/Pak® is a kit containing ADP (adenosine-5'-diphosphate), soluble Collagen and Ristocetin. All reagents are lyophilized for long term storage and stability.

INTENDED USE
BETA/Pak is a convenience kit containing a combination of routine platelet aggregation reagents used to elicit responses in Platelet Rich Plasma as well as an agglutination response that may be induced by the ristocetin reagent.

PRINCIPLE
ADP and Collagen (Type I) induce characteristic patterns of platelet aggregation in normal platelet rich plasma. Platelets from patients with various acquired and inherited platelet function disorders exhibit abnormal responses to these reagents.[8,10,11]

In the presence of the Ristocetin Cofactor Assay, Ristocetin induces agglutination of platelets. This response is decreased or absent in platelet rich plasma from patients with von Willebrand Syndrome.[15]

PRECAUTIONS
BETA/Pak reagents are for *PROFESSIONAL LABORATORY USE ONLY AND IN-VITRO DIAGNOSTIC USE ONLY AND NOT FOR INJECTION OR INGESTION.*

NOTE TO USER: Any serious incident that occurs in relation to this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

MATERIALS PROVIDED
1. ADP, 1 x 0.5mL. Store at 2° to 8° C prior to reconstitution.
2. Collagen (Type I), 1 x 0.5mL. Store at 2° to 8° C prior to reconstitution.
3. Ristocetin, 1 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 (0.45mL and 0.05mL volumes)
4. Disposable Stir bars
5. Aggregometer cuvettes

INSTRUMENTATION
BETA/Pak reagents 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, thrombocy topenia (<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 labora tory 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]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 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°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.
5. The platelet count of the PRP should be 250,000 ± 50,000/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.

1. Reconstitute a vial of ADP with 0.5mL purified water.
2. Reconstitute a vial of Collagen with 0.5mL purified water.
3. Reconstitute Ristocetin with 0.5mL purified water.

After reconstitution, mix all reagents well before using.

REAGENT STORAGE
The reconstituted ADP and Collagen are stable for 30 days when stored at 2°-8°C in their original tightly sealed container. Reconstituted Ristocetin is stable for 7 days when stored at 2°-8°C, or it may be frozen at -20°C for up to 8 weeks.

TEST PROCEDURE
Testing must be completed within 4 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 2 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 reagent directly into the platelet rich plasma. Do not allow reagent to run down the wall of the cuvette. The final concentration of each reagent in the test plasma is:
   ADP 2 x 10-5M
   Collagen 0.19mg/mL
   Ristocetin 1.5mg/mL
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]

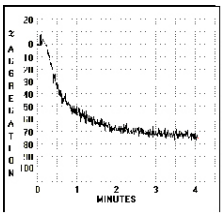
Prepare the diluted concentrations of ADP as follows:
1. Label 2 test tubes: 4 x 10^{-5} M and 2 x 10^{-5} M, see Table 1.
2. Add 0.4mL saline to the tube labeled 4x10^{-5}M, and add 0.2mL saline to the tube labeled 2x10^{-5}M.
3. To make the 4 x 10^{-5} M: add 0.1mL of the 2 x 10^{-4}M (from the reconstituted vial) to the tube labeled 4 x 10^{-5}M. Mix (a 1 to 5 dilution).
4. To make the 2 x 10^{-5}M: add 0.2mL of the 4 x 10^{-5}M (from the 4 x 10^{-5}M tube) to the tube labeled 2 x 10^{-5}M. Mix (a 1 to 2 dilution).
5. Additional dilutions may be achieved by using techniques as detailed above.

Table 1

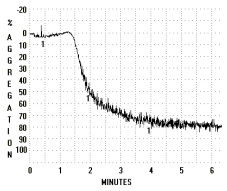
	Working Concentration	Final Concentration
Reconstituted	2 x 10^{-4}M	N/A
Normal	2 x 10^{-4}M	2 x 10^{-5} M
Biphasic	2 x 10^{-5} M up to 4 x 10^{-5} M	2 x 10^{-6} M up to 4 x 10^{-6} M

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

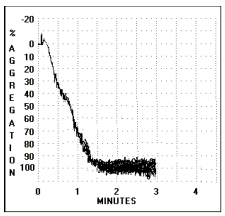
To assure proper instrument operation and reagent performance, a control specimen should be evaluated each day that tests are performed. The control specimen should be prepared in



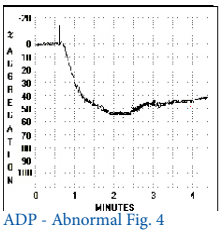
ADP - Normal Fig. 1



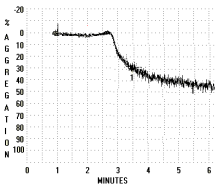
Collagen - Normal Fig. 2



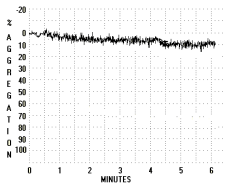
Ristocetin - Normal Fig. 3



ADP - Abnormal Fig. 4



Collagen - Abnormal Fig. 5



Ristocetin - Abnormal Fig. 6

RESULTS

Typical aggregation patterns are illustrated in Figures 1 - 6.

ADP, at final concentration of 2 x 10^{-5} M, will induce a large single wave of aggregation in normal platelet rich plasma. At a final concentration (in test) of 2 x 10^{-6} M to 4 x 10^{-6} M, 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 necleotides (storage pool) contained within the platelets.[9]

Collagen aggreagation occurs in a single wave following a lag phase during which the platelets adhere to the soluble collagen. During the phase, no aggregation is observed.

Ristocetin induced aggregation occurs as either a biphasic response or as one large wave of aggregation. The Primary wave is due to the agglutination of platelets by the von Willebrand factor in the presence of Ristocetin. The secondary wave is due to the release of endogenous ADP from the platelets.

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.	2.0x10^{-5} M	500µg/mL	0.19mg/mL	1.0x10^{-4} M
Lag Phase [sec]	<10	<=20	<60	0
Primary Slope	38-67	>20	35-67	7-34
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

Table 2: Aggregation Results Observed in Platelet Function Defects

Defect \ Reagent	ADP	Collagen	Ristocetin
ASPIRIN-LIKE	▼ or N	▼	▼ or N
THROMBASTHENIA	▼▼	▼	N
STORAGE POOL DISEASE	▼	▼	▼ or N
VON WILLEBRAND SYNDROME	N	N	▼▼
BERNARD-SOULIER SYNDROME	N	N	▼▼

▼ = Decreased aggregation due to decrease of absence of secondary wave.
▼▼= Decreased aggregation due to decrease or absence of primary and secondary wave.
N = Noraml Response

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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