AMERICAN BIOCHEMICAL & PHARMACEUTICALS LTD.

REF ABP-EPI-1
3 X 0.5mL, Lyophilized
Epinephrine

abp
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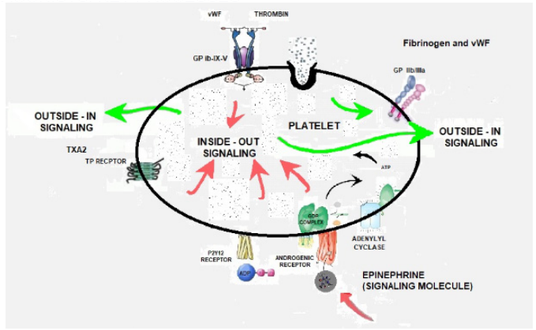
PRODUCT DESCRIPTION
Epinephrine Reagent is a lyophilized formulation of Adrenaline. The molecular weight is 183.207 and the working concentration is 1.0 mM.

INTENDED USE
Epinephrine is a weak agonist. The intended uses for Epinephrine Reagent are to detect platelet hyper-reactivity, the evaluation of qualitative platelet dysfunctions, and response to the presence of certain drugs, particularly those affecting platelet chloride transport, as well as other anti-platelet agents.
Epinephrine is commonly used along with a panel of other agonists to detect and identify platelet dysfunctions.[1,2]

TEST PRINCIPLES
EPINEPHRINE SIGNALING ACTIVATES THE PLATELET, ENHANCES ACTIONS OF OTHER AGONISTS: KEY PROCESSES



In Light Transmission Aggregometry (LTA), adding an agonist to Platelet Rich Plasma will result in platelets activating, changing shape and aggregating. This results in an increase of the amount of light transmitted through the sample (% Aggregation).
Epinephrine is a weak agonist. It will generate a single wave of aggregation. If the concentration is sufficient to result in degranulation, a secondary wave, caused by the release of endogenous ADP will appear.[3,4,5]
Not all patients respond to Epinephrine.

MATERIALS PROVIDED
Epinephrine Reagent , 3 X 0.5 mL
Store at 2 – 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. 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
Epinephrine Reagent will perform as describe 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 Epinephrine 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 it original, tightly sealed container

REAGENT DISPOSAL
Epinephrine is an IVD product and not intended for injection

Biological products must be handled with all necessary precautions and considered potentially infectious material.[6]

Epinephrine Reagent must be handled with appropriate precautions, including wearing appropriate laboratory clothing, safety glasses and gloves. Avoid contact with skin and do not ingest.

Handle the reagents with care to avoid contamination and air exposure during use.

Waste should be disposed of in accordance with laboratory policy and applicable local regulations.

PROFESSIONAL LABORATORY USE ONLY

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

EXPECTED VALUES* (test time 6 minutes)

EPINEPHRINE	WORKING CONCENTRATION	FINAL CONCENTRATION
	1.0mM	100.0 µM

LAG PHASE	PRIMARY SLOPE	FINAL AGGREGATION @ 6 MINUTES (%)	BIPHASIC AGGREGATION	AUC@6 MINUTES
0 sec	7 - 45	54 - 101	YES	

* Multiple reports indicate that between 20 and 50% of the normal population will generate a primary aggregation wave when epinephrine is used to stimulate platelets.[7]

RESULTS
Typical Epinephrine aggregation patterns are illustrated in Figures 1 and 2. Epinephrine will induce two distinct waves of aggregation in normal platelet rich plasma.[3,4]

Figure 1

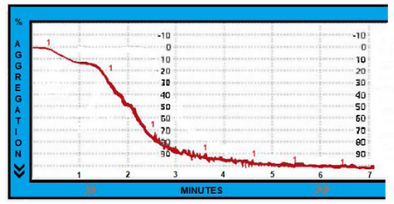
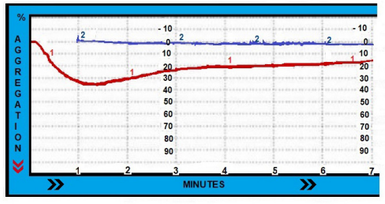


Figure 2



Note: Not all normal patients respond to Epinephrine Reagent.

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 @ 6minutes, 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.[8,9,10]

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.[16]

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.[11,12]

LIMITATIONS

Epinephrine does not work well when used for impedance aggregometry.
Spurious results may occur when the PRP platelet count is less than 75,000 platelets/cumm.

PRP that has not been held at room temperature for thirty minutes prior to testing may give erroneous results.

TEST PREPARATIONS

PATIENT PREPARATION[5,8,10,17]

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 collections.

SPECIMEN COLLECTION[6.7.8.9.10]

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 gauge 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.

15 - 28°C

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[9,11,13,14]

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 a nominal platelet count 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.

EPINEPHRINE RESPONSES

CONDITION	EPINEPHRINE RESPONSE
Glanzmann's Thrombasthenia	Absent
Bernard Soulier Syndrome	Normal
von Willebrand Disease	Normal
vWD Type 2b	Normal
Storage Pool Disease	Decreased
Myeloproliferative Disease	Decreased or Absent
Uremia	Normal or Decreased
Quebec Platelet Disorder	Absent
Aspirin	Normal or Decreased
P2Y12 Antagonist	Normal

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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