

PAK[®] 2-LE

INTENDED USE

PAK[®]2-LE is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies to HLA class I antigens and to epitopes on the platelet glycoproteins IIb/IIIa, Ia/IIa, and Ib/IX.

For *In Vitro* Diagnostic Use.

SUMMARY OF EXPLANATION

The existence of platelet-specific antigens on various platelet glycoproteins has been described by many investigators.^{1,2,3,4,5,6} Antibodies to platelet-specific or HLA class I antigens due to pregnancy or transfusion can result in immune destruction of transfused platelets.^{7,8,9} Confirming the presence of these antibodies in patient sera can be helpful in the search for potentially compatible blood products.

PAK[®]2-LE Solid Phase ELISA microwells provide monoclonal-captured platelet glycoproteins IIb/IIIa and Ia/IIa obtained from group O donors of known platelet types. HLA class I and platelet glycoprotein Ib/IX are provided as affinity purified glycoproteins. The test is designed to detect and differentiate between antibodies to HLA class I and platelet-specific antigens. The configuration of the wells can be found on the Recording Sheet.

PRINCIPLE OF THE PROCEDURE

Patient serum or plasma is added to microwells coated with platelet and HLA glycoproteins allowing antibody, if present, to bind. Unbound antibodies are then washed away. An alkaline phosphatase labeled anti-human globulin reagent (Anti-IgG) is added to the wells and incubated. The unbound Anti-IgG is washed away and the substrate PNPP (p-nitrophenyl phosphate) is added. After a 30 minute incubation period, the reaction is stopped by a sodium hydroxide solution. The optical density of the color that develops is measured in a spectrophotometer.

REAGENTS

Maximum number of tests per kit: 11

All reagents should be stored as directed by the label.

- | | |
|------------|--|
| MS | 1. Microwells: Flat-bottom microwell strips to which platelet and HLA glycoproteins have been immobilized.
3-1x8 GREEN strips containing HLA class I glycoproteins
3-1x8 BLUE strips containing GPIIb/IIIa glycoproteins
3-1x8 YELLOW strips containing GPIb/IX glycoproteins
3-1x8 WHITE strips containing GPIa/IIa glycoproteins
The microwells are color coded and enclosed in a resealable foil pouch. Ready for use. |
| TCW | 2. Concentrated Wash (10x): Tris (hydroxymethyl) aminomethane buffered solution containing sodium chloride and Tween 20. 1% sodium azide. Dilute with deionized or distilled water before use. Store Working Wash solution up to 48 hours at room temperature or up to seven days at 2 to 8°C. |
| SD | 3. Specimen Diluent: Phosphate buffered saline solution containing bovine albumin and mouse serum. 0.1% sodium azide. Ready for use. |
| SB | 4. Substrate Buffer: This solution contains diethanolamine and magnesium chloride. 0.02% sodium azide. Ready for use. Protect from light. |
| SS | 5. Stopping Solution: 3 M Sodium Hydroxide. Ready for use. Use with care. |
| AG | 6. Conjugate: Alkaline phosphatase conjugated goat affinity purified antibody to human immunoglobulin G (IgG). 0.1% sodium azide. Dilute in Specimen Diluent before use. |
| PN | 7. PNPP (p-nitrophenyl phosphate) Substrate: Crystalline powder. Reconstitute with deionized or distilled water and dilute in Substrate Buffer before use. Protect from light. |
| PC | 8. Positive Serum Control: Human Serum. 0.1% sodium azide. Dilute in Specimen Diluent before use. |

NC

9. Negative Serum Control: Human Serum. 0.1% sodium azide. Dilute in Specimen Diluent before use.

PS

10. Plate Sealers.

PRECAUTIONS

- Do not use reagents that are turbid or contaminated.
- Care **MUST** be taken to avoid contamination of Specimen Diluent and Conjugate. Inadvertent contamination of these reagents with human serum or plasma will result in the neutralization of the Conjugate and subsequently to test failure.
- Do not use reagents beyond their expiration date.
- Microwells and reagents contained in the kit are not to be used in conjunction with any other test system.
- Substitution of components other than those provided in this kit may lead to inconsistent or erroneous results.
- Discard any unused portions of diluted Conjugate, diluted Positive and Negative Controls, and diluted and reconstituted PNPP reagent after each run.
- When making dilutions, follow pipet manufacturer's instructions for appropriate dispensing and rinsing techniques.
- The enzyme substrate reaction which occurs in the final incubation is temperature sensitive and should be performed in a controlled area at 22 to 25°C.
- Due to variations in instruments or consistently higher or lower room temperatures, it may be necessary for the laboratory to establish a slightly longer or shorter incubation time in order to consistently achieve valid control results. Because the temperature of the final incubation can affect control values, it is important to periodically monitor the room temperature incubation.

CAUTION

- All human serum used in the Positive and Negative Controls for this product has been tested and found negative for antibody to HIV, HCV and HBsAg by FDA approved methods. No test method, however, can offer complete assurance that HIV, Hepatitis C virus, Hepatitis B virus or other infectious agents are absent. Therefore, these materials should be handled as potentially infectious.
- Some of the reagents supplied with this kit contain sodium azide as a preservative.
WARNING: Sodium azide reacts with lead and copper plumbing forming highly explosive metal azides. When discarded in a sink, the sink should be flushed with a large volume of water to prevent azide buildup. Sodium azide is a poison and is toxic if ingested.
- Stopping Solution (NaOH) is corrosive. Avoid contact with skin and eyes. Spills should be cleaned up immediately.
- Discard all components when completed according to local regulations.

SPECIMEN COLLECTION

Blood should be collected in ACD or EDTA (plasma) or without anticoagulant (serum) using aseptic technique and should be tested while still fresh to minimize the chance of obtaining false positive or false negative reactions due to improper storage or contamination of the specimen. Samples that cannot be tested immediately should be stored at 2 to 8°C for no longer than 48 hours or frozen. Samples frozen at -20°C or below remain in good condition for several years (2-3 years). However, in order to avoid the deleterious effect of repeated freeze/thaw cycles, it is recommended that samples should be aliquoted in small volumes and then stored frozen. Avoid frost-free freezers.

Serum or plasma should be separated from red cells when stored or shipped.

Particulates or aggregates in the sample can cause false positive results or poor duplicate values. Samples containing particulate matter should be clarified by centrifugation prior to testing.

Only whole human serum or plasma is suitable for this assay. Prior dilution of samples in anything other than normal, ELISA negative human serum could affect the results.

Microbially contaminated, hemolyzed, lipemic, icteric or heat-inactivated samples may give inconsistent test results and should be avoided.

PROCEDURE

Materials Provided:

Vials may contain more reagent than described on the labels. Be sure to measure the reagent with an appropriate device when making dilutions.

1. 12 – 1 x 8 Color-coded Microwell Strips with holder

2. 1 x 50 mL Concentrated Wash
3. 1 x 14 mL Specimen Diluent
4. 1 x 14 mL Substrate Buffer
5. 1 x 14 mL Stopping Solution
6. 1 x 80 μ L Anti-Human IgG Conjugate
7. 3 x 50 mg PNPP Substrate
8. 1 x 0.7 mL Positive Serum Control
9. 1 x 0.7 mL Negative Serum Control
10. 6 Plate Sealers

Additional Materials Required:

1. Test tubes for patient sample and control dilutions and for reagent dilutions
2. Transfer pipets
3. Adjustable micropipets to deliver 10 – 100 μ L and 100 – 1,000 μ L and disposable tips
4. Timer
5. Microplate reader capable of measuring OD at 405 or 410 and 490 nm
6. Deionized or distilled water
7. Absorbent paper towels
8. Microplate washer or device
9. Centrifuge capable of separating serum or plasma from patient samples
10. 37°C waterbath or incubator

Test Procedure

1. Bring all reagents to room temperature.
2. Make Working Wash solution by diluting Concentrated Wash. Add 1 volume of Concentrated Wash to 9 volumes of deionized or distilled water. Mix well.
3. Determine the number of patient samples to be tested. Using the Recording Sheet, assign each sample to a location consisting of two (duplicate) rows. Record the identity of each sample on the Recording Sheet.

PREPARE SAMPLES AND CONTROLS

4. Dilute as follows and mix well:

	Volume Specimen Diluent	Volume sample
PC	150 μ L	50 μ L
NC	300 μ L	100 μ L
Patient Sample	450 μ L	150 μ L

5. Remove microwell frame from pouch. Promptly remove and reseal unneeded strips in the protective pouch.

NOTE: Only one frame is provided in the kit. Do not discard until all strips have been used.
Be sure that the order of the strips in the frame matches the order on the Recording Sheet.

NOTE: Orient the frame with A1 in the top left corner. Be sure that all strips are properly seated and snapped into their frame. Label or number each strip to avoid errors. Maintain the same plate orientation throughout the assay.

6. Add 300 μ L of Working Wash solution to all wells and allow to stand at room temperature for 5-10 minutes.
7. Aspirate or decant forcefully and invert on absorbent toweling to prevent drying.
8. Add 50 μ L of the appropriate diluted control or sample to the wells as designated on the Recording Sheet.

NOTE: Do not add samples or reagents to blank wells.

NOTE: Only one set of controls is required per frame. If more than one color set is tested, LABEL EACH STRIP TO AVOID ERRORS.

9. Seal the microwells with a plate sealer and incubate for 30-35 minutes in a 37°C waterbath. If a dry incubator is used instead, increase time by 10 minutes.

10. Dilute the Conjugate 1 to 100 in Specimen Diluent. Use a polypropylene container.

Strips:	4 – 1 x 8	12 – 1 x 8
AG	20 µL	60 µL
SD	2.0 mL	6.0 mL

NOTE: Conjugate is viscous. Prime tip 2-3 times in Conjugate before dispensing and rinse after addition to Specimen Diluent. Mix well.

11 WASH STEP:

- a) Aspirate or decant contents of each well and blot on absorbent toweling.
- b) Add 300 µL Working Wash solution.
- c) Aspirate or decant.
- d) Repeat steps b + c for a total of 3 or 4 washes.
- e) Vigorously decant to remove all residual wash solution. Invert on absorbent toweling to prevent drying.

NOTE: It is important to completely remove all wash solution after the final wash.

12. Add 50 µL of diluted Conjugate (made in a previous step) to all wells EXCEPT those designated as BLANKS.
13. Seal the microwells with a plate sealer and incubate for 30-35 minutes in a 37°C waterbath. If a dry incubator is used instead, increase time by 10 minutes.
14. Dissolve PNPP Substrate by adding 0.5 mL deionized or distilled water to the vial. Replace stopper and mix well. Protect from light until use.
15. Dilute the PNPP 1 to 100 in the Substrate Buffer.

Strips:	4 – 1 x 8	12 – 1 x 8
PN	40 µL	120 µL
SB	4.0 mL	12.0 mL

Mix well. Protect from light until use.

16. WASH STEP:

- a) Aspirate or decant contents of each well and blot on absorbent toweling.
- b) Add 300 µL Working Wash solution.
- c) Aspirate or decant.
- d) Repeat steps b + c for a total of 3 or 4 washes.
- e) Vigorously decant to remove all residual wash solution. Invert on absorbent toweling to prevent drying.

Proceed promptly through next three steps.

17. Add 100 µL of the diluted PNPP solution to all the wells EXCEPT those designated as BLANKS.
18. Allow the microwells to stand in the dark for 30 minutes at ROOM TEMPERATURE (22 to 25°C).

NOTE: Incubation time and temperature after the addition of PNPP is critical. DO NOT vary the established incubation time or temperature. For consistency, begin timing promptly after addition of the reagent to the first well.

19. Stop the reaction by adding 100 µL of Stopping Solution to each well in the same sequence as the addition of substrate. Add 200 µL of Stopping Solution to the blank wells.
20. Read the absorbance (OD) of each well at 405 or 410 nm using a reference filter of 490 nm. If the results cannot be read immediately, return the wells to a dark location for up to 30 minutes.
21. Subtract the values obtained in the blank wells from all sample and control wells. Many ELISA readers are programmed to automatically perform this step.
22. Record the results on the Recording Sheet.

QUALITY CONTROL

Quality control of PAK[®]2-LE is built into the test system by the inclusion of Positive and Negative Serum Controls. These controls should be included with each test run to help determine if technical errors or reagent failures have occurred.

Criteria for a valid test:

	Negative Control	Positive Control
Mean OD	≤ 0.175 (IIb/IIIa)	≥ 1.500 (HLA row)

OD readings obtained from duplicate test results should fall within 20% of the mean of the two values. Samples whose results are outside of this limit should be re-tested.

NOTE: Poor duplicates can be the result of reagent or sample omission, uneven addition of reagents, uneven temperature during incubations, stray light during the final incubation or cross-well contamination. Failure to test in duplicate may lead to acceptance of erroneous results.

INTERPRETATION OF TEST RESULTS

Test results showing OD values equal to or greater than 2X the value obtained for the negative controls of the corresponding glycoprotein are regarded as positive results.

LIMITATIONS

Erroneous results can occur from bacterial contamination of test materials, inadequate incubation periods, inadequate washing or decanting of test wells, exposure of substrate to stray light, omission of test reagents, exposure to higher or lower than prescribed temperature requirements, or omission of steps.

The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased non-specific binding and produce false-positives in this assay.

Test results that do not fit a pattern of allo-antibody specificity are considered indeterminate. These samples may be re-drawn and/or re-tested, or tested by another method such as GTI MACE[®] or MAIPA.

The results of this assay should not be used as the sole basis for a clinical decision.

Some low titer, low avidity antibodies may not be detected using this assay.

This product does not detect IgM or IgA antibodies.

Some non-cytotoxic HLA antibodies may be detected by this technique that do not react in the lymphocytotoxicity assay (LCA).

Antibodies to low incidence HLA class I antigens may not be detected using this product.

SPECIFIC PERFORMANCE CHARACTERISTICS

When properly stored and used according to the procedures described above, this product can detect (IgG) antibodies to HLA class I antigens and to platelet-specific antigens on the immobilized glycoproteins.

To ensure suitable reactivity and specificity, each lot of PAK[®]2-LE is tested prior to release with samples known to contain antibodies reactive with the glycoproteins identified on the Recording Sheet as well as samples known to be free of such antibodies.

Performance Evaluation

		Comparative Method		
		Positive	Negative	Total
PAK [®] 2-LE	Positive	97	5	102
	Negative	13*	266	279
	Total	110	271	381

Agreement: 95.3%

Co-positivity: 88.2%

Co-negativity: 98.2%

Comparative Method: Reverse Passive Hemagglutination Assay

* Because the comparative method incorporates whole, intact platelets as the target for antibody detection, it can give positive results with any antigen expressed on platelets.¹⁰ The targets for antibody detection in PAK[®]2-LE are the individual glycoproteins, and thus will not detect cold agglutinins or antibodies to red cell antigens such as Lewis.

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PAK[®]2-LE

- FOR *IN VITRO* DIAGNOSTIC USE
- STORE AT 2 to 8°C



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