

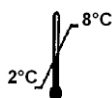


## Pak Lx<sup>®</sup> Assay

REF

PLX RUO

RUO

MATCHIT! Platelet Antibody RUO software v1.1 ( [REF 888622 RUO](#))MATCHIT! Platelet Antibody RUO software User Manual ( [REF LC1371RUO](#))MATCHIT! Platelet Antibody RUO software Quick Reference Guide ( [REF LC1374RUO](#))

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#### Restricted Use Label License:

By opening the packaging containing this Kit (which contains fluorescently labeled microsphere beads authorized by Luminex Corporation) or using this Kit in any manner, you are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return this Kit for a full refund prior to using it in any manner.

You, the customer, acquire the right under Luminex Corporation's patent rights, if any, to use this Kit or any portion of this Kit, including without limitation the microsphere beads contained herein, only with Luminex Corporation's laser based fluorescent analytical test instrumentation marketed under the name Luminex instrument

## DEFINITION OF SYMBOLS

(Product Labels and Supplemental Documents)

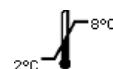
For Research Use Only. Not for use in diagnostic procedures.



Catalog Number



Temperature range (storage)



Keep away from light



**Caution!**  
Consult  
Accompanying  
Documents



Warning



Consult  
instructions  
for use



Manufacturer



Use By Date



Batch Code



Dilute Before  
Use



Sufficient for N  
tests



## ASSAY DESCRIPTION

Pak Lx Assay is a qualitative immunoassay for use on the Luminex instrument(s).

## SUMMARY AND EXPLANATION

Platelets express a variety of polymorphic proteins. The polymorphic changes in the proteins, while not affecting protein function, might become the targets for antibodies as a result of pregnancy or transfusion. The presence of antibodies that bind to platelet glycoproteins is associated with life-threatening bleeding disorders such as refractoriness to platelet transfusions (PR), post-transfusion purpura (PTP), and fetal and neonatal alloimmune thrombocytopenia (FNAITP).<sup>1-13</sup>

The glycoproteins (GP) targeted by antibodies include GPIIb/IIIa, GPIb/IX and GPIa/IIa, GPIV, and Class I Human Leukocyte Antigens (HLA). The epitopes found on GPIIb/IIIa, GPIb/IX, and GPIa/IIa have been characterized into Human Platelet Antigen (HPA) systems of two alleles each (alleles "a" and "b"). HPA-1, HPA-3, and HPA-4 are all located on GPIIb/IIIa. HPA-2 is located on GPIb/IX and HPA-5 is located on GPIa/IIa.<sup>14-16</sup> The epitopes of GPIV are presented only as isoantigens. In other words, the development of antibodies to GPIV occurs in individuals who do not have or have significantly reduced levels of GPIV expression, but have become exposed to GPIV.<sup>17</sup> Class I HLA is encoded by numerous alleles expressing a large number of diverse epitopes. The vast majority of antibodies produced in response to pregnancy or transfusion will be reactive with the highly polymorphic Class I HLA.<sup>18</sup>

## PRINCIPLE OF THE PROCEDURE

The Pak Lx Beads are reconstituted and incubated with serum sample at room temperature. The beads are then washed to remove unbound antibody. An anti-Human IgG antibody conjugated to phycoerythrin (PE) is then added. After further incubation at room temperature, the reaction mixture is diluted and analyzed on the Luminex instrument(s). The signal intensity from each bead is compared to the signal intensity of three negative control beads included in the bead preparation to determine if the bead is positive or negative for bound antibody.

## REAGENTS

Maximum number of tests per kit:

- **PLX RUO: 16 tests per kit**

All reagents should be stored as directed by the label.

|             |            |                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|-------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|             | <b>REF</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>PLXB</b> | 403620     | Lyophilized Bead Blend: A blend of beads to which HPA, GPIV and Class I HLA glycoproteins have been immobilized along with four control beads. The beads are lyophilized in a phosphate-based buffer containing bovine serum albumin and 0.02% methylisothiazolone and 0.02% bromonitrodioxane as preservatives. LIGHT SENSITIVE. Keep out of direct light for extended periods of time (3 hours or less). Store at 2 to 8°C in the dark, in kit box. |
| <b>PLBD</b> | 405160     | Bead Diluent: A phosphate-based buffer containing NaCl, Tween-20, ProClin 300, bovine serum albumin and mouse serum. Store at 2 to 8°C.                                                                                                                                                                                                                                                                                                               |

|             |        |                                                                                                                                                                                                                                                                                                                                                                       |
|-------------|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>CCX</b>  | 405159 | Conjugate Concentrate: Goat anti-Human IgG conjugated to phycoerythrin provided in a phosphate-based storage buffer containing NaCl, Tween-20, <0.1 % sodium azide, and bovine serum albumin. LIGHT SENSITIVE. Keep out of direct light for extended periods of time (2 hours or less). Store at 2 to 8°C in the dark. Dilute 1:10 in Conjugate Diluent prior to use. |
| <b>CDX</b>  | 403586 | Conjugate Diluent: A phosphate-based buffer containing NaCl, Tween-20, 0.1% sodium azide, bovine serum albumin and mouse serum. Store at 2 to 8°C.                                                                                                                                                                                                                    |
| <b>LMWB</b> | 405315 | Wash Buffer: A phosphate-based buffer containing NaCl, Tween-20, 0.1% sodium azide, and bovine serum albumin. Store at 2 to 8°C.                                                                                                                                                                                                                                      |
| <b>PCX</b>  | 403595 | Positive Control Serum: This serum or sera blend is obtained from individual(s) with known antibodies to HPA-1a with or without antibodies to Class I HLA. Store at 2 to 8°C. Contains 0.1% sodium azide.                                                                                                                                                             |
| <b>NCX</b>  | 403592 | Negative Control Serum: This serum or sera blend is obtained from individual(s) with no known antibodies to HPA, GPIV and HLA. Store at 2 to 8°C. Contains 0.1% sodium azide.                                                                                                                                                                                         |

## PRECAUTIONS

- For Research Use Only. Not for use in diagnostic procedures.
- Do not use reagents that are turbid or contaminated.
- Do not use reagents beyond their expiration date.
- Discard all unused / diluted Positive and Negative Controls and Conjugate after use.
- 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.
- When making dilutions, follow pipet manufacturer's instructions for appropriate dispensing and rinsing techniques.
- Care MUST be taken to avoid contamination of Conjugate Diluent or the anti-Human IgG reagent. Inadvertent contamination of these reagents with human serum will result in the neutralization of anti-Human IgG and subsequently result in test failure.
- Care must be taken during pipetting into the filter plate, that beads do not stick to the side of the microplate wells being pipetted, while being careful not to touch the membrane with the tip. Contacting the membrane with the pipet tip can lead to puncture of the membrane and subsequent failure of the assay.
- Care must be taken to ensure, during incubation steps, that the beads are not splashing and sticking to the sides of the wells. The positive and negative controls must be included with each test to help determine if technical error or reagent failures have occurred.
- Care must be taken to control vacuum strength. Strong vacuum pressure can cause beads to stick to the membrane causing bead count failure.

## 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 reagents contain sodium azide as a preservative, which may react with lead and copper plumbing to form explosive metal azides. Use large amounts of water when discarding materials down a sink.
- Dispose of all materials after use according to local regulations.

## INSTRUMENTATION

The Luminex 100/200 or Luminex FLEXMAP 3D instrument running the Luminex xPONENT software is to be used for performing the Pak Lx Assay. The Luminex 100/200 instrument or Luminex FLEXMAP 3D system is based on the principles of flow cytometry and includes the Luminex analyzer, the Luminex XY plate handling platform, and the Luminex SD sheath fluid delivery system, software and PC. The Luminex 100/200 or Luminex FLEXMAP 3D System is the combination of three core xMAP Technologies. The first is microspheres, a family of 100 fluorescently dyed polystyrene microspheres that act as both the identifier and the solid surface to build the assay. The second is a flow cytometry-based instrument, the Luminex analyzer, which integrates key detection components such as lasers, optics, fluidics and high-speed digital signal processors. The third component is the software, which is designed for protocol-based data acquisition.

Information on the features of the software and the instrument installation, operation, maintenance and safety are provided in the Luminex 100/200 or Luminex FLEXMAP 3D User Manual. The user should contact the Luminex Corporation for specific services regarding the Luminex 100/200 or Luminex FLEXMAP 3D instrument system.

The assay files required to use the Pak Lx Assay are provided by Immucor [GTI Diagnostics, Inc](#) on their [customer center](#) for download by the user.

The user should contact Immucor GTI Diagnostics for any product related concerns for Pak Lx Assay and the MATCHIT!® Platelet Antibody RUO Software.

## SPECIMEN COLLECTION AND STORAGE

Collect blood without anticoagulant using aseptic technique. Process blood while still fresh to minimize the chance of obtaining false-positive or false-negative reactions due to improper storage or contamination of the specimen.

**Only serum is suitable for this assay.**

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

Samples containing particulate matter should be clarified by centrifugation prior to testing.

Serum 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 or below –20°C remain in good condition for up to 2 years. Avoid frost-free freezers. Avoid repeated freezing and thawing of the serum samples.

## PROCEDURE

### Materials Provided:

(See REAGENTS section for more specific information)

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

1. 3 x Lyophilized Bead Blend
2. 1 x 850 µL Bead Diluent
3. 1 x 120 µL Conjugate Concentrate
4. 1 x 1.2 mL Conjugate Diluent
5. 1 x 50 mL Wash Buffer
6. 1 x 80 µL Positive Control Serum
7. 1 x 80 µL Negative Control Serum

### Additional Materials Required:

(as listed or equivalent)

1. 5 µL to 50 µL adjustable pipets with appropriate pipet tips
2. 250 µL multichannel pipet with matching tips and buffer trough
3. 1.5 mL microcentrifuge tubes for conjugate dilutions
4. Test tubes
5. Timer
6. Marking pen
7. Plate sealers
8. Luminex Sheath Fluid  
Lifecodes **REF** 628005 (1X); Luminex **REF** 40-50018 (20X) or Luminex Sheath Fluid Plus, Lifecodes **REF** 628010
9. Luminex Calibration Kits (Luminex 100/200 Calibration Kit, Luminex 100/200 Performance Verification Kit)  
Lifecodes **REF** 628018 and **REF** 628019 respectively or FLEXMAP 3D Calibration Kits (Luminex FLEXMAP 3D Calibration Kit, Luminex FLEXMAP 3D Performance Verification Kit, LIFECODES Cat No. **REF** 888307 and **REF** 888308 respectively)
10. Distilled water
11. Rotator or vortex with plate adapter
12. Millipore Multiscreen filter plates  
**REF** 888633 Millipore MSBVN1210/MSBVN1250/MABVN1210/MABVN1250
13. Multiscreen vacuum manifold  
**REF** 888315 Millipore MAVM0960R; Qiagen 19504; Pall 5017
14. Microcentrifuge
15. Lot specific Luminex Acquisition Template file; available on customer center
  - LXT file for use with the Luminex 100/200 or Luminex FLEXMAP 3D
16. Lot specific Electronic Data Sheet (EDS) file; available on customer center
17. Luminex 100/200 instrument or Luminex FLEXMAP 3D with xPONENT software
18. **PFS** Plate Format Sheet
19. **PACD** MATCHIT! Platelet Antibody RUO Software Installation USB  
MATCHIT! Platelet Antibody RUO Software v1.1  
MATCHIT! Platelet Antibody RUO software User Manual; also available on customer center  
MATCHIT! Platelet Antibody RUO software Quick Reference Guide; also available on customer center

## Test Procedure

1. Download the lot specific Luminex acquisition template (LXT file) from the [customer center](#) and save it on the computer connected to the Luminex instrument. Note the location of the saved file. This file will be imported to the Luminex instrument in a later step.
2. Bring all reagents except the Conjugate Concentrate and the Conjugate Diluent to room temperature (21 to 24°C) prior to use.
3. Reconstitute the Lyophilized Bead Blend as noted in Table 1 below.

The number of vials provided in Table 1 includes enough volume to run one positive and one negative control along with the number of samples listed in column 1.

Table 1

| Number of samples | Vial(s) of Lyophilized Bead Blend |
|-------------------|-----------------------------------|
| 1-4               | 1                                 |
| 5-10              | 2                                 |
| 11-16             | 3                                 |

- a. Add 275 µL of Bead Diluent to each vial of lyophilized beads. Allow the reconstituted vial to sit at room-temperature for at least 5 minutes.
- b. **Do not** mix or vortex the Lyophilized Beads with the Bead Diluent at this time.
  - Reconstituted vials may be stored at 2-8°C, in the dark for up to 15 days.

**CAUTION:** DO NOT POOL VIALS RECONSTITUTED ON DIFFERENT DAYS INTO ONE.

**CAUTION:** DO NOT VORTEX THE BEADS AT ANY TIME DURING THE ASSAY TO AVOID FROTHING.

4. Prepare samples by vortexing. Centrifuge at  $\geq 10,000 \times g$  for at least 60 seconds to settle any insoluble matter before use.
5. Record sample information on the Plate Format Sheet. The plate format sheet is available for download from the [customer center](#).
6. Cover the unassigned wells of the filter plate with a plate sealer.
7. Add 250 µL of wash buffer to the assigned wells.
  - a. Allow the wash buffer to sit in the wells for at least 3 minutes.
  - b. Remove the wash buffer by gentle aspiration using the vacuum manifold.  
(See manufacturer's recommendations for proper use.)
8. Mix the reconstituted beads by pipetting up and down about 15 to 20 times taking care to **avoid any frothing**.
9. Add 40 µL of Reconstituted Beads to each test well of the filter plate. Ensure that the Reconstituted Beads are mixed during bead addition to the filter plate. Pipet up and down 2-3 times after delivery to every 2-3 wells.

**CAUTION:** It is important to keep the beads resuspended to ensure uniform bead addition to the wells. Failure to mix beads intermittently will cause beads to settle towards the bottom of the vial. This will result in differential amount of beads being dispensed into wells which may adversely affect run-times and analysis of results.
10. Add 10 µL of patient serum or control serum referring to the plate layout sheet.
11. Return unused portions of control sera and beads to storage at 2 to 8°C in the dark for future use.
12. Cover the plate with a plate sealer.
13. Incubate for 60 minutes at room temperature (21 to 24°C) in the dark on a rotating platform (approximately 200 rotations per minute) or a vortex shaker with a plate adaptor set at a speed that will allow proper mixing without splashing of the samples.
  - a. During this incubation, bring the Conjugate Concentrate and Conjugate Diluent to room temperature (21 to 24°C).
  - b. During this Incubation, warm up and prepare the Luminex instrument.

- c. Set up the assay on the Luminex instrument by using the lot specific LXT file (acquisition template) downloaded from the customer center. Refer to the Luminex software manual on how to add/import a template file into the software.

**NOTE:** When using the Automated Batch Setup feature, refer to the MATCHIT! Platelet Antibody RUO software User Manual which is available on the Platelet Antibody Software Installation USB PACD and on the customer center.

**CAUTION:** Failure to use the correct LXT file will result in the collection of data that is out of sequence and will incorrectly or incompletely capture the assay data from the Luminex instrument.

14. Prepare diluted Conjugate Concentrate by mixing the volumes of Conjugate Concentrate (CCX) with Conjugate Diluent (CDX) as listed in Table 2 below. The volumes shown in Table 2 below include enough volume to run one positive and one negative control along with the number of samples listed in column 1.

- a. Cover the diluted conjugate with foil or store in the dark at room temperature until used.
- b. Return the unused portion of Conjugate Concentrate and Conjugate Diluent to storage at 2 to 8°C in the dark for future use.

Table 2

| Number of Samples | Volume of CCX (μL) | Volume of CDX (μL) |
|-------------------|--------------------|--------------------|
| 1                 | 20.0               | 180.0              |
| 2                 | 25.0               | 225.0              |
| 3                 | 30.0               | 270.0              |
| 4                 | 35.0               | 315.0              |
| 5                 | 40.0               | 360.0              |
| 6                 | 45.0               | 405.0              |
| 7                 | 50.0               | 450.0              |
| 8                 | 55.0               | 495.0              |
| 9                 | 60.0               | 540.0              |
| 10                | 65.0               | 585.0              |
| 11                | 70.0               | 630.0              |
| 12                | 75.0               | 675.0              |
| 13                | 80.0               | 720.0              |
| 14                | 85.0               | 765.0              |
| 15                | 90.0               | 810.0              |
| 16                | 95.0               | 855.0              |

15. After the 60 minute incubation, remove the plate sealer and add 200 μL of Wash Buffer to each well. Remove the wash buffer by gentle aspiration using the vacuum manifold.

**CAUTION:** Use of excessive vacuum strength will cause beads to stick to the membrane and can result in an assay failure. Apply **the minimum** vacuum pressure required to aspirate samples. Refer to the Manufacturer's instructions for specific use of the filter plates and vacuum manifold.

16. Add 250 μL of Wash Buffer to each well, aspirate. Repeat two more times.

**CAUTION:** Failure to wash completely may reduce the ability of the conjugate to detect IgG bound to sensitized beads.

17. Add 50 μL of diluted conjugate to each well. Cover with plate sealer. Incubate for 30 minutes at room temperature (21 to 24°C), in the dark on a rotating platform (approximately 200 rotations per minute) or a vortex shaker with a plate adaptor set at a speed that will allow proper mixing without splashing of the samples. Do not save the diluted conjugate for future use.

18. Remove the plate sealer. Add 150 μL of Wash Buffer to each well containing the sample. Return the unused portion of Wash Buffer to storage at 2 to 8°C for future use.

19. Immediately collect data with Luminex 100/200 or Luminex FLEXMAP 3D instrument using the manufacturer's instructions.

**NOTE:** A minimum of 60 events must be collected per bead region.

## QUALITY CONTROL

The Positive and Negative Control Sera serve as external assay controls and must be included with each test run. The Positive Control Serum is prepared from available serum donors and may change with each lot of Pak Lx. The Positive Control Serum should have a positive reported result for those antibodies reported on the Certificate of Quality Control. The Negative Control Serum should have a negative reported result for any HPA, GPIV or HLA Class I bead. If these requirements are not met, the affected samples are to be considered invalid.

Quality control of the Pak Lx assay is built into the test system by the inclusion of four control beads, (one positive control bead and three negative control beads). The Positive Control Bead is coated with human IgG and should yield MFI values  $\geq 10,000$  with the Negative Control Sera. If values of  $< 10,000$  MFI are obtained with the Negative Control serum, the assay may be insufficiently washed or the conjugate may be compromised. Samples may show a wide range of reactivity with the Positive Control Bead, but should produce a signal of  $\geq 3500$  MFI. If these requirements are not met, the affected samples are to be considered invalid.

The interpretation of sample data is based on having a minimum number of each bead collected during an assay. When too few bead events are acquired for a sample, a "Bead Failure" error is noted and all antibody results for the affected sample are to be considered invalid.

Please see the troubleshooting section of these instructions for further details.

## INTERPRETATION OF RESULTS

**Interpretation by software:** The MATCHIT! Platelet Antibody RUO software (v1.1 with current applicable patches available at the Immucor Customer Center) is required for the interpretation of the results. For the analysis, a lot-specific EDS file is required and is available for download from the [customer center](#).

**Determination of individual bead reactivity:** To determine if an antigen-specific bead is positive or negative, the MFI of the antigen-specific bead is divided by the MFI for each Negative Control Bead (CON1, CON2, CON3), producing three ratios for each antigen-specific bead. Note: when the MFI of any CON bead is less than the Minimum Cutoff (MC), then the MC is used in the calculation. From the three calculated ratios, a predetermined ratio, the Background Adjustment Factor (BAF) is subtracted from each antigen-specific bead/con combination to obtain three Adjusted Ratios.

- A positive value for two or more of the three Adjusted Ratios indicates a positive bead reaction.
- A negative value for two or more of the three Adjusted Ratios indicates a negative bead reaction.

**Interpretation of results:** Refer to the MATCHIT! Software report for the results. The table below lists the possible results that could be obtained when testing any given sample.

| Antigen         | GPIV       | HLA Class I | HPA-1, -3, -4<br>(GPIIb-IIIa) | HPA-2<br>(GPIb-IX)          | HPA-5<br>(GPIa-IIa)         |
|-----------------|------------|-------------|-------------------------------|-----------------------------|-----------------------------|
| Possible result | Pos<br>Neg | Pos<br>Neg  | Reactive<br>Neg               | Pos<br>Neg<br>Indeterminate | Pos<br>Neg<br>Indeterminate |

Results for HLA Class I, GPIV, HPA-2 and HPA-5 are directly reported by the MATCHIT! software. Any indeterminate results for HPA-2 and HPA-5 should be repeated using the Pak Lx Assay or using an alternate method. To determine HPA-1, -3 and -4 reactivity, use the following table:

| HPA coated beads                                                 | Pattern of Bead Reactivity** |                   |                              |        |                              |                                  |
|------------------------------------------------------------------|------------------------------|-------------------|------------------------------|--------|------------------------------|----------------------------------|
| HPA – 1a-3a-4a                                                   | Pos                          | Neg               | Pos                          | Neg    | Pos                          | Neg                              |
| HPA – 1a-3b-4a                                                   | Pos                          | Neg               | Neg                          | Pos    | Pos                          | Neg                              |
| HPA – 1b-3a-4a                                                   | Neg                          | Pos               | Pos                          | Neg    | Pos                          | Neg                              |
| HPA – 1b-3b-4a                                                   | Neg                          | Pos               | Neg                          | Pos    | Pos                          | Neg                              |
| HPA – 1ab-3ab-4a                                                 | Pos*                         | Pos*              | Pos*                         | Pos*   | Pos*                         | Neg                              |
| HPA - 1a-3ab-4b                                                  | Pos                          | Neg               | Pos*                         | Pos*   | Neg*                         | Pos                              |
| Interpretation of results                                        | Antibodies to HPA-1 detected |                   | Antibodies to HPA-3 detected |        | Antibodies to HPA-4 detected |                                  |
| This pattern may mask the presence of an antibody reactive with: | HPA-4b                       | HPA-4b not masked | HPA-4b                       | HPA-4b | HPA-1a, -1b, -3a, -3b        | HPA-1a, -1b, -3a, -3b not masked |

\* Heterozygous beads may be positive or negative depending on the titer of the antibody in the sample or the level of background on the negative control beads.

\*\*Any pattern not represented in the table above should be considered an indeterminate result and may be due to the presence of auto-antibodies or a combination of auto- and allo- antibodies. This may also include reactivity to polymorphic variants not identified in the assay.

## LIMITATIONS

- This product has not been designed to detect antibodies of the IgA or IgM class of immunoglobulin.
- A positive test only indicates the presence of antibodies specific for HLA Class I, GPIV, or HPA-1, HPA-2, HPA-3, HPA-4 and HPA-5, and is not intended to diagnose a clinical condition.<sup>19-21</sup>
- The HPA systems occur in different frequencies in different populations. The higher frequency HPA systems are deemed to be “public” while the lower frequency HPA systems are deemed to be “private”. The Pak Lx assay uses glycoproteins captured from donors that have only been typed for the public HPA systems: HPA-1, -2, -3, -4, and -5. Three of these public HPA systems (HPA-1, -3, -4) are displayed on the same glycoprotein, GPIIb/IIIa.<sup>1,5,11,14</sup> Serum samples may have antibodies to one or more of these public HPA systems, and the presence of antibodies to one HPA system may mask the presence of antibodies to other systems. For example, the presence of a high titer antibody reactive with one HPA-4 epitope may mask the presence of lower titer antibodies reactive with one of the HPA-1 epitopes. Please see the section of these instructions labeled Interpretation of Results for further detail.

## TROUBLESHOOTING

| PROBLEM                                                                                          | POSSIBLE CAUSE                                                                                         | SOLUTION                                                                                                                                                                                             |
|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Positive control Serum shows additional Antibody reactivity not indicated on the QC certificate  | Positive Control contaminated with another sample                                                      | User to employ good laboratory technique including proper aliquoting methods to eliminate carryover of samples to adjacent wells; Repeat the assay                                                   |
|                                                                                                  | Poor washing                                                                                           | Repeat assay following wash instructions                                                                                                                                                             |
| Negative Control Serum shows Antibody reactivity                                                 | Contaminated reagents                                                                                  | User to employ good laboratory technique including proper aliquoting methods to eliminate carryover of samples to adjacent wells; Repeat the assay                                                   |
|                                                                                                  | Poor washing                                                                                           | Repeat the assay following wash instructions                                                                                                                                                         |
| For the Negative Control Serum, the MFI value on the Positive Control Bead (Probe 77) is <10,000 | Photobleached conjugate                                                                                | Use new kit; Repeat the assay                                                                                                                                                                        |
|                                                                                                  | Poor washing                                                                                           | Repeat the assay following wash instructions                                                                                                                                                         |
| For test samples, the MFI value on the Positive Control Bead (Probe 77) is <3,500                | Photobleached conjugate                                                                                | Use new kit; Repeat the assay                                                                                                                                                                        |
|                                                                                                  | Poor washing                                                                                           | Retest the sample following wash instructions                                                                                                                                                        |
| MFI values on antigen beads are approximately 30 MFI while Positive Control bead MFI is > 10,000 | Sample not added                                                                                       | Retest the sample                                                                                                                                                                                    |
| Bead Failure (insufficient bead events counted) (<60 events collected)                           | Beads not mixed well or resuspended                                                                    | Mix well with pipet to completely resuspend the beads; Retest the sample                                                                                                                             |
|                                                                                                  | Instrument failures - out of calibration                                                               | See Instrument Manual; Repeat the assay                                                                                                                                                              |
|                                                                                                  | Instrument failures - sample flow blocked                                                              | See Instrument Manual; Repeat the assay                                                                                                                                                              |
|                                                                                                  | Photobleached beads                                                                                    | Use new kit; Repeat the assay                                                                                                                                                                        |
|                                                                                                  | Vacuum pressure too strong/beads stuck to membrane                                                     | Reduce vacuum strength; Retest the sample                                                                                                                                                            |
| Uninterpretable results                                                                          | Use of incorrect acquisition template (LXT) file.                                                      | Check to see if the lot number in the LXT file used for the acquisition of data matches the Pak Lx kit used for testing. If incorrect, repeat the assay and acquire data using the correct LXT file. |
|                                                                                                  | Use of incorrect EDS (BAF) file for performing the analysis by MATCHIT! Platelet Antibody RUO software | Check to see if the lot number in the EDS file used for the analysis of data matches the Pak Lx kit used for testing. If incorrect, repeat the data analysis using the correct EDS file.             |

## PERFORMANCE CHARACTERISTICS

Specific performance characteristics have not been determined.

## REFERENCES

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|         |                                                                           |
|---------|---------------------------------------------------------------------------|
| Warning | Warning                                                                   |
| H317    | May cause an allergic skin reaction                                       |
| P261    | Avoid breathing dust/fume/gas/mist/vapours/spray                          |
| P272    | Contaminated work clothing should not be allowed out of the workplace     |
| P280    | Wear protective gloves/protective clothing/eye protection/face protection |

|             |                                                                    |
|-------------|--------------------------------------------------------------------|
| P302 + P352 | IF ON SKIN: Wash with plenty of soap and water.                    |
| P333 + P313 | If skin irritation or rash occurs: Get medical advice/attention.   |
| P501        | Dispose of contents/container to an approved waste disposal plant. |