AbScreen HLA class I

**Intended Use**
AbScreen HLA class I is a solid phase Enzyme Linked ImmunoSorbent Assay (ELISA) for in-vitro detection of IgG antibodies to HLA class I antigens.

**Summary and Explanation**
HLA is a major antigenic system in determining the survival of transplant allografts or transfused platelets in sensitized individuals (1). HLA class I antibodies can be formed after blood transfusions, after organ transplantations and during pregnancy (2). The highly polymorphic human leukocyte (HLA) class I antigens are widely distributed on all nucleated cells. Platelets, although not nucleated, are fragments of nucleated megakaryocytes and carry the HLA class I antigens (3). AbScreen HLA class I provides affinity-purified HLA class I glycoproteins obtained from platelets of White, Black, and Hispanic blood donors. The purified glycoproteins are immobilized in microwells. Using AbScreen HLA class I, anti HLA class I antibodies from transfused, transplanted or pre-transplanted patients can be identified rapidly.

**Principle of the Enzyme Immunosorbent Assay**
AbScreen HLA class I is a solid phase enzyme linked immunosorbent assay (ELISA). Microtiter plates are coated with highly purified HLA class I antigens from a large pool of human platelets. If the sample being tested contains specific antibodies against HLA class I, they will bind to the antigen in the wells of the microtiter plate. Unbound antibodies are removed in a washing step. The resulting antibody-antigen complex is detected using a specific enzyme-labelled antibody directed against human IgG (conjugate). The presence of bound antibodies is demonstrated by adding a chromogenic substrate (PNPP) which results in a coloured product. The reaction is stopped and interpreted by means of an ELISA reader.

**Statement of Precautions**
For In Vitro Diagnostic Use.
- The test must be performed by well-trained and authorised laboratory technicians.
- All human serum used in the Positive and Negative Controls for this product has been tested and found to be non-reactive for HBsAg, anti-HCV and anti-HIV-1/2 antibodies. However, all products of human origin should be considered to be potential transmitters of hepatitis, HIV or other infectious agents. Appropriate safety measures are recommended.
- Do not use reagents past the expiration date printed on the label.
- Do not use reagents with turbidity or any evidence of microbial contamination.
- Do not mix reagents of different lots.
- Discard all unused diluted solutions from substrate [PNPP], conjugate CONJ, positive CONTROL and negative control [CONTROL] after each run.
- Avoid contamination of Sample Dilution Buffer [SAMP|BUF] and conjugate CONJ with human serum. This could be result in the neutralization of the conjugate and subsequently to the test failure.
- The substrate incubation is temperature sensitive and should be performed in a controlled area at 22-25°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.

**Warnings**
- Some of the reagents supplied with this kit contain sodium azide (NaN₃) as a preservative.
- Sodium azide reacts with lead and copper plumbing forming very toxic gas. Wear protective gloves/protective clothing/eye protection/face protection, IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
- Stop Solution (STOP) may be corrosive to metals. It causes severe skin burn and eye damage. Do not breathe vapours. Wear protective gloves/protective clothing/eye protection/face protection. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. Dispose of contents/container to hazardous or special waste collection point.
- Acquiring to good laboratory practice wear gloves, laboratory coat and safety glasses.
- Waste information is provided in the safety data sheet.

**Storage and Expiration**
The reagents are stable up to the stated expiry dates on the individual labels when stored at 2-8°C. All the reagents are lot specific and cannot be used with kits of other lots.

**Kit Contents**
The content is sufficient for a maximum of 88 samples.
- 2 microtiter plates [MTP], each containing 12 green strips, coated with HLA class I glycoproteins.
- 125 µl Wash Buffer (10 x) [BUF|10x] Tris(hydroxymethyl)-aminomethane, Tween 20, 1% NaN₃.
- 30 µl Substrate Dilution Buffer [SUB|BUF] (ready for use): Diethanolamine, MgCl₂, 0.02%NaN₃.
- 30 µl Sample Dilution Buffer (SAMP|BUF) [READY|BUF] (ready for use): Phosphate Buffered Saline (PBS), bovine serum albumin, 0.1% NaN₃.
- 30 µl Stop Solution (STOP) (ready for use): 3 M sodium hydroxide.
- 160 µl anti-IgG Conjugate [CONJ] (100 x): alkaline phosphatase conjugated, 0.1% NaN₃.
- 12500 mg Substrate (PNPP) [SUB|PNPP] p-nitrophenyl phosphate, crystalline.
- 720 µl AbScreen HLA class I Positive Control [CONTROL+] 0.1% NaN₃.
- 2x700 µl AbScreen HLA class I Negative Control [CONTROL-] 0.1% NaN₃.
- 24 self-adhesive transparent sealers.
- Reactivity sheets.

**Specimen collection and Preparation**
Fresh serum samples should be used. Heat inactivated samples or samples with microbial contamination may give nonspecific reactions and should not be used. Lipoaemic or hemolysed samples should be avoided.
- Serum should be stored at 2-8°C for no longer than 48 hours. For a longer period (up to 2 years) it is possible to store the serum samples frozen at -20°C or below.
- Avoid repeated freezing and thawing of the samples.
- Samples containing particulate or aggregates could produce false positive results or poor duplicate values and should be clarified by centrifugation prior to testing.

**Procedure**
Materials required, but not supplied with the kit
- ELISA reader (filter 405 or 410 nm, reference filter 490 or 492 nm).
- Incubator for 37 ± 1°C or waterbath (37 ± 1°C).
- ELISA Washer (not absolutely necessary).
- Micropipettes with adjustable volumes.
- 8-channel-pipette.
- Tubes for sample dilutions.
- Centrifuge for separating serum from patient samples.
- Distilled water.

**Preparation of the Reagents and Samples**
- Bring all reagents to room temperature before use.
- Dilute Wash Buffer (10x) [BUF|10x] 1:10 with distilled water. Mix well! Diluted Wash Buffer can be stored at 2-8°C for 7 days or up to 48 hours at room temperature.
- Dilute the samples, negative and positive controls with Sample Dilution Buffer [SAMP|BUF] (ready for use) as follows (mix thoroughly):

| Sample     | 100 µl sample | +100 µl Sample Dilution Buffer [SAMP|BUF] |
|------------|---------------|------------------------------------------|
| AbScreen HLA class I Positive Control | 75 µl Positive Control [CONTROL+] | +75 µl Sample Dilution Buffer [SAMP|BUF] |
| AbScreen HLA class I Negative Control | 110 µl Negative Control [CONTROL-] | +110 µl Sample Dilution Buffer [SAMP|BUF] |

| AbScreen HLA class I Positive Control | 75 µl Positive Control [CONTROL+] | +75 µl Sample Dilution Buffer [SAMP|BUF] |
| AbScreen HLA class I Negative Control | 110 µl Negative Control [CONTROL-] | +110 µl Sample Dilution Buffer [SAMP|BUF] |

When testing only 1 strip mix 75 µl of HLA class I Negative Control [CONTROL-] with 75 µl of Sample Dilution Buffer [SAMP|BUF].
Test Procedure
1. Remove the required strips from the pouch and promptly reseal unused strips into the pouch. Each sample and each control are tested in duplicate. For each assay 2 blanks, 4 negative controls and 2 positive controls are needed. In this case at least 44 patient samples can be performed on 1 microtiter plate [MTP].
2. Add 300 µl diluted Wash Buffer to all wells and incubate for 5 - 10 minutes at room temperature (22-25°C).
3. Decant the contents of each well by inverting the plate and blotting on absorbent material to remove any residual fluid. Do not allow the plate to dry out.
4. Add 50 µl of diluted Negative Control according to the reactivity sheet directory included.
5. Add 50 µl of diluted Positive Control according to the reactivity sheet included.
   - Caution: Discard unused diluted controls.
6. Add 50 µl of each diluted sample according to the reactivity sheet included.
7. Cover the plate [MTP] with a self-adhesive sealer and incubate at 37°C for 40-45 minutes in an incubator or 30-35 minutes in a waterbath (37°C).
8. Decant or aspirate the liquid from the wells and add 250 µl diluted Wash Buffer to each well. Do not discard the liquid and blot on absorbent material to remove any residual fluid. Repeat for a total of four washes. Alternatively a Bio-Rad washer can be used for this step.
   - After the last wash step the wells should be completely empty.
   - Do not allow the wells to dry out.
9. For 12 strips [MTP] mix 60 µl Conjugate [CONJ] to 6 ml of Sample Dilution Buffer [SAMP|BUF]. (When only 1 strip is used, mix 10 µl of Conjugate [CONJ] to 1 ml of Sample Dilution Buffer [SAMP|BUF].) Mix well.
10. Add 50 µl of fresh diluted Conjugate to all wells, except the Blank wells.
11. Add the plate [MTP] with a self-adhesive sealer and incubate at 37°C for 40-45 minutes in an incubator or 30-35 minutes in a waterbath (37°C).
12. Dissolve 50 mg of Substrate PNPP [SUB|PNPP] (1 bottle) with 500 µl of distilled water. The substrate solution must always be prepared fresh. For 12 strips [MTP], mix 120 µl of substrate solution [SUB|PNPP] with 12 ml Substrate Dilution Buffer. Mix well. (For 1 strip, mix 20 µl substrate solution with 2 ml Substrate Dilution Buffer.)
13. Wash 4x (see step 8).
   - After the last wash step the wells should be completely empty.
   - Do not allow the wells to dry out.
14. Add 100 µl of diluted substrate solution to all wells, except the Blank wells. Discard the unused substrate solution!
15. Incubate 30 minutes at room temperature (22-25°C) in the dark.
   - Caution: The incubation time and temperature is critical after the addition of substrate. Do not exceed the defined incubation time!
16. Add 100 µl of Stop Solution (STOP) to all the wells (including the blank wells).
17. Add an additional 100 µl of Stop Solution (STOP) to the Blank wells.
18. Measure the absorbance after addition of the stop solution at 405 or 410 nm using an ELISA reader. If the results cannot be read immediately, return the wells to a dark location for up to 30 minutes.
   - Confirm that the blanks are in the proper position and correspond to the Blanks designated on the ELISA reader.
19. Record the results on the Reactivity Sheet.

Quality Control
Each test run must include positive and negative controls which must give following values:
- Manual: Negative control mean O.D.: 0.040 - 0.150
- Positive control mean O.D. ≥ 1.500

Automated on Quickstep:
Negatives control mean O.D.: 0.030 - 0.200
Positive control mean O.D. ≥ 1.000

If mean OD values were obtained out of this specifications (OOS), the test run is invalid and has to be repeated. For the interpretation of the results consider a grey zone of 15% (OD value cutoff ≤ 15%)
(grey zone < OD value cutoff). Samples which have an OD value within the grey zone have to be tested with the AbIdent HLA class I ELISA.

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
Sample results showing O.D. values twice the mean value of the negative controls are regarded as positive results. The duplicates should fall within 20% of the mean of the two values. Otherwise the test should be repeated.

Limitations
Erroneous test results can occur from bacterial contamination of test materials, inadequate incubation periods, inadequate incubation temperature and inadequate washing of wells.

The presence of immune complexes or other immune aggregates in the samples can produce false positive results.

Samples from patients with autoimmune disorders or receiving immunoglobulin therapy have not been tested. Such samples may give false positive or false negative results.

Some low titer antibodies to HLA class I antigens or antibodies to low frequency antigens of the HLA class I system may not be detected and can give false negative results.

IgM, IgA antibodies and HLA class II antibodies will not be detected.

Non-cytotoxic IgG antibodies can give positive results in AbScreen HLA class I, that are not detected by lymphocytoxicity test (LCT). Conversely cytotoxic IgM antibodies will be negative by AbScreen HLA class I test. Therefore AbScreen HLA class I should be combined with a lymphocytoxicity test (for example: Bio-Rad and Lymphoscreen ABC [60, REF 823 300]).

Specific Performance Characteristics
When properly stored and used according to the procedures described above, AbScreen HLA class I can detect IgG antibodies to HLA class I antigens. A pool of at least 300 donors from different origins is used in order to assure that each antigen is represented in the pool as follows:

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Comparative Method for Performance Evaluation:
Lymphocytoxicity Assay (LCA)

Comparative Method
AbScreen HLA class I

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Agreement: 93.3 %
Co-positivity: 87.6 %
Co-negativity: 97.5 %
Specificity: (Sensitivity)

References
3. Harrison J, Navarrete C: Selection of Platelet Donors and Provision of HLA-
   55:192-196.
5. DIN EN ISO 980 Graphic symbols for use in the labelling of medical devices.