UK

AbIdent HLA class I

Package size

REF[3]	823420
MTP	Microtiter plate
CONJ	Anti-IgG Conjugate
BUF 10x	Wash Buffer (10x)
SUB PNPP	Substrate (PNPP)
SUB BUF	Substrate Dilution Buffer
SAMP BUF	Sample Dilution Buffer
CONTROL +	Positive Control
CONTROL -	Negative Control
STOP	Stop Solution



Harmful



Toxic



Irritant



Corrosive

Intended use

AbIdent HLA class I is a solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) for in-vitro detection of IgG antibodies (PRA) 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 (4). AbIdent HLA class I provides HLA class I glycoproteins obtained from platelets of 40 donors, each immobilized in different microwells.
UsingAbIdent 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

AbIdent HLA class I is a solid phase enzyme linked immunosorbent assay (ELISA). Microtiter plates are coated with 40 different human HLA class I glycoproteins. The HLA class I type of each donor and the number of the wells containing each antigen can be found on the reactivity sheet. 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.
AbIdent HLA class I is designed to be used as a companion product to AbScreen HLA class I. Samples previously identified as positive in AbScreen HLA class I can be further tested on AbIdent HLA class I to determine a PRA value and/or specificity.

Statement of Precautions

IVD 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 using FDA licensed test kits. 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.
- Discard all unused diluted solutions from reconstituted substrate SUB PNPP, conjugate CONJ, positive CONTROL + and negative control CONTROL - after each run.
- All the reagents are lot specific and can not be used with kits of other lots.
- 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 (NaN_3) 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.

- Stop Solution STOP (NaOH) is corrosive. Avoid contact with skin and eyes. Spills should be cleaned up immediately.
- According to good laboratory practice wear gloves, laboratory coat and safety glasses.
- Discard all components when completed according to local regulations.

Storage and Expiration

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8°C.

Kit contents

The content is sufficient for a maximum of 10 samples.

- 5 Microtiter plates MTP, each 12 colour coded strips, coated with HLA class I glycoproteins
- 125 ml Wash Buffer (10 x) BUF 10x: Tris(hydroximethyl)-aminomethane, Tween 20, 1% NaN_3
- 60 ml Substrate Dilution Buffer SUB BUF (ready for use): Diethanolamine, $MgCl_2$, 0.02%NaN_3
- 50 ml Sample Dilution Buffer SAMP BUF (ready for use): Phosphate Buffered Saline (PBS), bovine albumin and mouse serum, 0.1% NaN_3
- 50 ml Stop Solution STOP (ready for use): 3 M sodium hydroxide
- 350 µl anti-IgG Conjugate CONJ (100 x): alkaline phosphatase conjugated, 0.1% NaN_3
- 5x50 mg Substrate (PNPP) SUB PNPP: p-nitrophenyl phosphate, crystalline powder
- 300 µl AbIdent HLA class I Positive Control CONTROL +: 0.1% NaN_3
- 700 µl AbIdent HLA class I Negative Control CONTROL -: 0.1% NaN_3
- 10 self-adhesive transparent sealers
- Recording 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. Lipaemic or hemolyzed 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 particulates 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)
- Micropipetes with adjustable volumes
- 8-channel-pipete
- 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	550 µl sample + 1650 µl Sample Dilution Buffer SAMP BUF
AbIdent HLA class I Positive Control	20 µl Positive Control CONTROL + + 60 µl Sample Dilution Buffer SAMP BUF
AbIdent HLA class I Negative Control	60 µl Negative Control CONTROL - + 180 µl Sample Dilution Buffer SAMP BUF

Please find multiple pipetting scheme in figure 1.

Test Procedure

1. Remove the plate from the pouch and promptly remove and reseal unused strips into the pouch. Each sample requires 6 colour coded strips in the following order: yellow – green – blue – violet – red – orange.
   ➲ When placed in the frame, the strips must have the colour coded tab at the top.
   For each sample to be tested, 4 negative controls (NC), 1 positive control (PC) and 2 blanks are required. Please see pipetting scheme for one sample:

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	yellow 1	green 2	blue 3	violet 4	red 5	orange 6
A	Sample	Sample	Sample	Sample	Sample	NC
B	Sample	Sample	Sample	Sample	Sample	NC
C	Sample	Sample	Sample	Sample	Sample	NC
D	Sample	Sample	Sample	Sample	Sample	NC
E	Sample	Sample	Sample	Sample	Sample	PC
F	Sample	Sample	Sample	Sample	Sample	Sample/ NAW
G	Sample	Sample	Sample	Sample	Sample	Blank
H	Sample	Sample	Sample	Sample	Sample	Blank

2. Add 250 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 plate and blot on absorbent material to remove any residual fluid. Do not allow the plate to dry out.
4. Add 50 µl per well of diluted Negative Control according to the pipetting scheme.
5. Add 50 µl per well of diluted Positive Control according to the pipetting scheme.
   - Caution: Discard unused diluted controls.
6. Add 50 µl of each diluted sample into wells A1-H5 and F6 according to the pipetting scheme.
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 liquid from the wells and add 250 µl diluted Wash Buffer to each well. Decant or aspirate the liquid and blot on absorbent material to remove any residual fluid. Repeat for a total of four washes. Alternatively an ELISA 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 1 sample mix 30 µl Conjugate CONJ to 3 ml of Sample Dilution Buffer SAMP BUF.
10. Add 50 µl of **fresh** diluted Conjugate to all wells, **except the Blank wells**.
11. 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).
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 1 sample, mix 50 µl of substrate solution with 5 ml Substrate Dilution Buffer SUB BUF. Mix well. Protect from light until use.
13. Wash 4x (see step 8.).
    - After the last washing 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 are critical after the addition of substrate. Do not exceed the recommended 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.
      The ELISA reader should be programmed in such a way that the mean Blank value is subtracted from all other O.D. values.
19. Record the results on the Recording Sheet.

Figure 1: Dilution of reagents with higher sample numbers

Reagent	2 Samples (1 plate)	4 Samples (2 plates)	6 Samples (3 plates)	8 Samples (4 plates)	10 Samples (5 plates)
BUF 10x	28 ml	53 ml	78 ml	103 ml	**125 ml**
Distilled Water	252 ml	477 ml	702 ml	927 ml	**1125 ml**
AbIdent HLA class I CONTROL -	150 µl	250 µl	350 µl	450 µl	**550 µl**
SAMP BUF	450 µl	750 µl	1050 µl	1350 µl	**1650 µl**
AbIdent HLA class I CONTROL +	35 µl	60 µl	85 µl	115 µl	**135 µl**
SAMP BUF	105 µl	180 µl	255 µl	345 µl	**405 µl**
CONJ	55 µl	110 µl	165 µl	220 µl	275 µl
SAMP BUF	5,5 ml	11 ml	16,5 ml	22 ml	27,5 ml
SUB PNPP	110 µl	220 µl	330 µl	440 µl	550 µl
SUB BUF	11 ml	22 ml	33 ml	44 ml	55 ml

Quality Control
Each test run must include positive and negative controls.
Criteria for a valid test:

Mean OD value of the Negative Control ≤ 0.250
Mean OD value of the Positive Control ≥ 0.900

Interpretation of test results
The cutoff values are be calculated as described below. The background factor (BAF) of each well is lot specific. Please see Recording Sheet or lot information disk.

Calculation of the cutoff:

Mean negative control O.D. values x 2 x BAF = Cutoff for the well

Test results showing O.D. values equal or greater than the cutoff are regarded as positive reactions.
Test results showing O.D. values lower than the cutoff are regarded as negative reactions.

Calculation of the PRA:

$$\frac{\text{Number of positive reactions} \times 100}{40} = \%\text{PRA}$$

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.
The No Antigen Well (NAW) does not contain HLA glycoproteins or antibodies. This well is used for detection of non-specific binding. The O.D. value of this well should be equal to the negative controls. If O.D. value of the NAW well is higher than the negative control cutoff, the test should be repeated by LCT (e.g. Biotest Lymphoscreen ABC 60, art.no.823 300).
Samples from patients with autoimmune disorders or receiving immunoglobulin therapy have not been tested. Such samples may give false positive or false negative results.
IgM antibodies, IgA antibodies and HLA class II antibodies will not be detected. Non-HLA lymphocytotoxic antibodies will not be detected.
Non-cytotoxic IgG antibodies can may be detected in the Biotest AbIdent HLA class I, that are not detected by lymphocytotoxicity test (LCT). Conversely cytotoxic IgM antibodies will be negative by the Biotest AbIdent HLA class I test.Therefore the Biotest AbIdent HLA class I test should be combined with a lymphocytotoxicity test (for example: Biotest Lymphoscreen ABC 60, art. no. 823 300).
Low titre or low avidity antibodies may not detected by Biotest AbIdent HLA class I. Non-HLA lymphocytotoxic antibodies and antibodies directed to HLA antigens not represented on the Biotest AbIdent system will not be detected.
The Biotest AbIdent HLA class I test should not be solely used for clinical decisions.

Specific Performance Characteristics
When properly stored and used according to the procedures described above, this product can detect IgG antibodies to the HLA class I antigens identified on the enclosed Reactivity Sheet. Each lot has been tested against antisera known to be positive as well as with sera known to be free of antibodies.

Comparative Method for Performance Evaluation: ELISA method for PRA Determination
Sample Size:n=21

Comparative Method

AbIdent HLA class I	Positive	Negative
Positive	14	0
Negative	0	7

Agreement: 100 % Co-positivity: (Specificity) 100 % Co-negativity: (Sensitivity) 100 %

References

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Bio-Rad Medical Diagnostics GmbH
Industriestr. 1, D-63303 Dreieich, Germany
Tel.: +49-6103-3130-0, Fax: +49-6103-3130-724
www.bio-rad.com, techsupport.bmd@bio-rad.com

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