

Quik-ID® CLASS II

INTENDED USE

Quik-ID® Class II is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect Panel Reactive IgG antibodies (PRA) to HLA class II antigens.

For *In Vitro* Diagnostic Use.

SUMMARY AND EXPLANATION

HLA is a major antigenic system in determining the survival of transplant allografts or transfused platelets in sensitized individuals.¹ HLA antibodies can be acquired through alloimmunization as a result of pregnancy, transfusion of blood products, or previous transplants. In general, alloimmunization leads to the production of HLA antibodies in approximately 33% of exposed individuals.²

Quik-ID® Class II Solid Phase ELISA provides HLA class II glycoproteins obtained from EBV transformed B lymphocyte cell lines, each immobilized in different microwells by means of a monoclonal antibody. The HLA class II type of each cell line and the number of wells containing each antigen can be found on the Recording Sheet.

Quik-ID® Class II Solid Phase ELISA is designed to be used as a companion product to B-SCREEN®. Samples previously identified as positive in the screening assay can be further tested on Quik-ID® Class II to determine a PRA value and/or specificity.

PRINCIPLE OF THE PROCEDURE

Patient serum is added to microwells coated with HLA class II 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: 10

All reagents should be stored as directed by the label.

- | | |
|------------|--|
| MS | 1. Microwells: Flat-bottom microwell strips to which HLA class II glycoproteins have been immobilized. The microwells are color coded and enclosed in resealable foil pouches. 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 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 of 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 without anticoagulant 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 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 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 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. 5 Microwell frames, each containing 10 – 1 x 8 color coded strips and a color key card
2. 1 x 125 mL Concentrated Wash
3. 1 x 50 mL Specimen Diluent
4. 1 x 60 mL Substrate Buffer
5. 1 x 50 mL Stopping Solution
6. 1 x 350 µL Anti-Human IgG Conjugate
7. 5 x 50 mg PNPP Substrate
8. 1 x 0.45 mL Positive Serum Control
9. 1 x 0.7 mL Negative Serum Control
10. 10 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 µL and 100 – 1,000 µL 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.

Reagents	1 Plate (2 Samples)	2 Plates (4 Samples)	3 Plates (6 Samples)	4 Plates (8 Samples)	5 Plates (10 Samples)
TCW	25 mL	50 mL	75 mL	100 mL	125 mL
DI Water	225 mL	450 mL	675 mL	900 mL	1,125 mL

3. Determine the number of patient samples to be tested.

PREPARE SAMPLES AND CONTROLS

4. Dilute as follows and mix well:

Patient Sample	425 µL
SD	1.275 mL

Reagents	1 Plate (2 Samples)	2 Plates (4 Samples)	3 Plates (6 Samples)	4 Plates (8 Samples)	5 Plates (10 Samples)
NC	120 µL	250 µL	350 µL	450 µL	550 µL
SD	360 µL	750 µL	1,050 µL	1.35 mL	1.65 mL
PC	60 µL	120 µL	180 µL	240 µL	300 µL
SD	180 µL	360 µL	540 µL	720 µL	900 µL

5. Each sample requires one set of color-coded strips. Promptly remove and reseal the unneeded strips in the protective pouch.

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.

Ensure that the rounded tab on each strip is at the bottom edge of the frame. Before each reagent addition, ensure that well A1 is in the top left-hand corner of the frame.

NOTE: Be sure that the order of the strips in the frame matches the order on the color key card.

6. Add 250 μL 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 μL of the appropriate diluted control or sample to the wells as designated in figure 1. Add patient serum to all numbered wells and to the NA and MO wells.

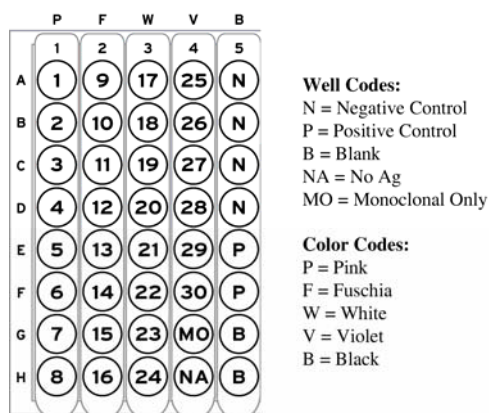


Figure 1

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

NOTE: If multiple patient samples are tested at the same time, 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.

Reagents	1 Plate (2 Samples)	2 Plates (4 Samples)	3 Plates (6 Samples)	4 Plates (8 Samples)	5 Plates (10 Samples)
AG	45 μL	90 μL	135 μL	180 μL	225 μL
SD	4.5 mL	9 mL	13.5 mL	18 mL	22.5 mL

NOTE: Conjugate is viscous. Prime pipet 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 250 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.

Reagents	1 Plate (2 Samples)	2 Plates (4 Samples)	3 Plates (6 Samples)	4 Plates (8 Samples)	5 Plates (10 Samples)
PN	90 µL	180 µL	270 µL	360 µL	450 µL
SB	9 mL	18 mL	27 mL	36 mL	45 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 250 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 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 Quik-ID® Class II is built into the test system by the inclusion of Positive and Negative Serum Controls. These controls should be included with each test sample to help determine if technical errors or reagent failures have occurred.

Criteria for a valid test:

	Negative Control	Positive Control
Mean OD	≤ 0.225	≥ 1.000

INTERPRETATION OF TEST RESULTS

Calculate the Cutoff value for each individual well as follows:

$$\begin{array}{l} \text{Mean of Neg. Control wells} \\ \text{(A, B, C, \& D of Black strip)} \end{array} \times 2 \times \begin{array}{l} \text{Background Adjustment Factor} \\ \text{(see Recording Sheet)} \end{array} = \begin{array}{l} \text{Cutoff Value} \\ \text{for the well} \end{array}$$

Test results showing OD values equal to or greater than the cutoff value are regarded as being positive results.

Calculate the percent PRA (Panel Reactive Antibody) as follows:

$$\% \text{ PRA} = \frac{\# \text{ of Positive Results}}{\# \text{ of wells containing HLA class II antigen}} \times 100$$

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.

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.

The No Antigen Well (well H of violet strip) does not contain monoclonal antibody or HLA glycoproteins and is used to detect non-specific binding.

The "Monoclonal Only" well (well G of violet strip) contains only murine monoclonal antibody. Reactivity in this well could be due to reactivity with the capture antibody of the test system, presence of antibodies to mouse IgG, or to immune complexes.

Patient samples that give higher values in the "No Antigen Well" and/or "Monoclonal Only Well" than the cutoff value of the negative control should be tested by another method.

Non-HLA lymphocytotoxic antibodies will not be detected using this assay.

This product does not detect IgM, IgA, or HLA class I antibodies.

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

This product is expected to detect HLA antibodies to antigens that are represented on the panel. (See lot specific Recording Sheet.) Antibodies to unrepresented antigens may not be detected.

SPECIFIC PERFORMANCE CHARACTERISTICS

When properly stored and used according to the procedures described above, this product can detect antibodies to the HLA class II antigens identified on the enclosed Recording Sheet.

To ensure suitable reactivity and specificity, each lot of Quik-ID® Class II is tested prior to release with samples known to contain alloantibodies reactive with the HLA class II antigens as well as samples known to be free of such antibodies.

Performance Evaluation

Comparative Method

Quik-ID® Class II		Positive	Negative	Total
		Positive	45	
	Negative	3	36	39
	Total	48	39	87

Agreement: 93.1 %

Co-positivity: 93.7 % Co-negativity: 92.3 %

Comparative Method: Bead Based Flow Cytometry

REFERENCES

1. Marsh SGE, Parham P, Barber LD, The HLA Facts Book. Academic Press 2000: 84-91.
2. Rodey Glenn E. HLA Beyond Tears. De Novo, Inc. 2000; 213.

U.S. Patent #6,046,013



GTi DIAGNOSTICS

Good science starts with people.™

Quik-ID® Class II

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

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