

MACE[®]2 (Modified Antigen Capture ELISA)

INTENDED USE

MACE[®]2 is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies to epitopes on the platelet glycoproteins Ia/IIa, Ib/IX, and IV.

For *In Vitro* Diagnostic Use

SUMMARY AND 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 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.

MACE[®]2 microwells provide immobilized monoclonal antibodies designed to capture platelet glycoproteins Ia/IIa, Ib/IX or IV. The assay is designed for use in detecting and differentiating between platelet-specific antibodies directed against donor or patient platelets.

PRINCIPLE OF THE PROCEDURE

Patient serum or plasma is incubated with intact platelets allowing for antibody, if present, to bind to the platelet glycoproteins. Unbound antibodies are washed from the platelets. The antibody-sensitized platelets are solubilized by the addition of a lysis buffer containing a non-ionic detergent. The platelet lysate containing soluble glycoproteins is transferred to microwells. This allows the platelet glycoproteins (sensitized or unsensitized with patient antibody) to be captured by immobilized monoclonal antibodies. Control samples are handled similarly. After a brief incubation period, unbound glycoproteins are 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. A positive result indicates the presence of glycoprotein-specific antibody on the captured GPIa/IIa, GPIb/IX, or GPIV.

REAGENTS

Maximum number of tests per kit: 30

All reagents should be stored as directed by the label.

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| MS | 1. Microwells: Flat-bottom microwell strips to which murine monoclonal antibodies specific to platelet glycoproteins Ia/IIa, Ib/IX, and GPIV have been immobilized.
4-1x8 PURPLE strips specific for GPIa/IIa,
4-1x8 ORANGE strips specific for GPIb/IX, and
4-1x8 BLACK strips for GPIV.
The microwell strips are 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. |
| TSD | 3. Specimen Diluent: Tris buffered saline solution containing sodium chloride. 0.05% 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. |

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| 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. Ready for use. |
| NC | 9. Negative Serum Control: Human Serum. 0.1% sodium azide. Ready for use. |
| CRP | 10. Cell Resuspension and Preservative Solution: Phosphate buffered saline solution, containing bovine albumin. 0.1% sodium azide. Ready for use. |
| CLB | 11. Cell Lysis Buffer (10x): Tris buffered saline solution containing a non-ionic detergent. 0.1% sodium azide. Dilute with deionized or distilled water before use. |
| NCP | 12. Normal Platelet Control: Dried pooled human platelets. Rehydrate before use with Cell Resuspension and Preservative Solution. |
| PS | 13. 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.
- Diluted Cell Lysis Buffer **MUST** be used on the day of preparation and not stored for future use.
- 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

Serum or Plasma:

Blood should be collected in ACD (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.

Serum or plasma 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.

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

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

Platelets:

- Platelet samples should be collected in CPD or ACD and tested within 7 days. When not tested immediately, platelets should be stored at room temperature (20 to 25°C). Platelet samples obtained from platelet concentrates should be used within prescribed dating period.
- 2 – 3 mL of platelet rich plasma ($\leq 400,000$ per μL) or 0.25 mL of platelet concentrate or 15 μL of a 50% suspension of washed platelets for each serum sample to be tested. Platelet-rich plasma can be prepared by centrifuge whole blood at $2000\times g$ for 3 minutes.
- Platelets should be centrifuged at a time and speed adequate to pellet the cells. Excessive centrifugation, however, is not recommended. Suggested centrifugation times are $580\times g$ for 20 minutes, $2,000\times g$ for 10 minutes, or $5,000\times g$ for 6 minutes. Each laboratory should establish the optimal times and speeds for the equipment in use.

Platelet Storage:

- Prepare a platelet button as described in step 5 below. Discard the supernatant and resuspend the platelet button in 0.25 mL of Cell Resuspension and Preservative Solution. This suspension may be stored at 2 to 8°C for up to 7 days.

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 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 μL Anti-Human IgG Conjugate
7. 4 x 50 mg PNPP Substrate
8. 1 x 0.7 mL Positive Serum Control
9. 1 x 0.7 mL Negative Serum Control
10. 2 Normal Platelet Control Vials (50 μL rehydrated)
11. 1 x 2.5 mL Cell Lysis Buffer
12. 1 x 50 mL Cell Resuspension and Preservative Solution
13. 8 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 platelets
10. 37°C waterbath or incubator
11. Microcentrifuge tubes
12. Microcentrifuge for pelleting platelets

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 one well on each strip. Record the identity of each sample on the Recording Sheet.

PREPARE SAMPLES AND CONTROLS

4. Prepare the positive and negative controls:
 - a) Rehydrate one vial of the dried Normal Platelet Control by adding 400 μL of Cell Resuspension and Preservative Solution.
 - b) Allow to stand for 10-30 minutes at room temperature.
 - c) Mix the rehydrated platelets well.
 - d) Centrifuge tubes to pellet cells
 - e) Decant supernatant and blot the tubes dry.
 - f) Add 50 μL of Cell Resuspension and Preservative Solution to the platelet button.
 - g) Mix well with the aid of a pipet tip to obtain a homogenous suspension. Transfer 15 μL of the platelet suspension into each of two clean microcentrifuge tubes.
 - h) Add 150 μL of Positive Serum Control to one of the tubes. Label as positive control. Add 150 μL of Negative Serum Control to the other tube. Label as negative control. **MIX WELL**

Prepare test platelets:

5. For each platelet sample to be tested, place 2 – 3 mL of platelet rich plasma (platelet count should not exceed 400,000 per μL) or 0.25 mL platelet concentrate in a microcentrifuge tube and centrifuge to obtain a platelet button. Discard supernatant plasma.
6. Add 400 μL of Cell Resuspension and Preservative Solution to the platelet button and **MIX WELL**. Centrifuge to obtain a platelet button.
7. Invert the tube and discard the supernatant fluid. Repeat steps 6 and 7 for a total of 3 to 4 washes. Following the last wash, blot tubes with an absorbent paper towel. Allow the supernatant to be drained away.
8. Estimate the volume of platelet button obtained. Make a 50% suspension of each platelet by adding an equal volume of Cell Resuspension and Preservative Solution to the platelet button. Mix well to obtain a homogenous suspension. Transfer 15 μL of the 50% Platelet Suspension to a clean labeled tube.
9. Add 150 μL of patient serum or plasma to each of the tubes containing a platelet test suspension prepared in the previous step. **MIX WELL** with the aid of a pipet.
10. Incubate the positive and negative control and test samples for 30 – 35 minutes at 37°C. If a dry incubator is used, increase the incubation time by 10 minutes.
11. Wash all test and control samples twice by adding 400 μL of Cell Resuspension and Preservative Solution to each tube and centrifuging to pellet the platelets. Discard supernatant fluid after each wash. Blot tubes with absorbent paper to remove all residual fluids, being careful not to disturb the platelet button after final wash.
12. Dilute Cell Lysis Buffer. Add 100 μL of Cell Lysis Buffer to 900 μL of deionized or distilled water. Prepare 1.0 mL diluted buffer for each three platelet samples to be lysed. **MIX WELL**.
13. To lyse the platelets, add 180 μL of diluted Cell Lysis Buffer (prepared in the previous step) to each test and control tube. **MIX WELL** with the aid of a pipet or vortex to obtain complete lysis. Promptly proceed to the assay. Platelet lysates should be tested as soon as possible.

PERFORMING THE ASSAY

14. 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.

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.

15. Add 50 μL of sensitized platelet lysate, negative control and positive control lysate to the wells designated on the Recording Sheet.

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

NOTE: LABEL EACH STRIP TO AVOID ERRORS.

16. 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.

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

Strip:	3 – 1 x 8	12 – 1 x 8
IgG	20 μL	60 μL
TSD	2 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

18. WASH STEP:

- Aspirate or decant contents of each well and blot on absorbent toweling.
- Add 300 μL Working Wash solution.
- Aspirate or decant.
- Repeat steps b + c for a total of 3 or 4 washes.
- 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.

19. Add 50 μL of diluted Conjugate (made in a previous step) to all wells EXCEPT those designated as BLANKS.

20. 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.

21. Dissolve PNPP Substrate by adding in 0.5 mL deionized or distilled water to the vial. Replace stopper and mix_well. Protect from light until use.

22. Dilute the PNPP 1 to 100 in the Substrate Buffer.

Strip:	3 – 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.

23. WASH STEP:

- Aspirate or decant contents of each well and blot on absorbent toweling.
- Add 300 μL Working Wash solution.
- 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.

- 24. Add 100 µL of the diluted PNPP solution to all the wells EXCEPT those designated as BLANKS.
- 25. 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.

- 26. 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.
- 27. 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.
- 28. Subtract the values obtained in the blank wells from all sample and control wells. Many ELISA readers are programmed to automatically perform this step.
- 29. Record the results on the Recording Sheet.

QUALITY CONTROL

Quality control of MACE[®]2 is built into the test system by the inclusion of Positive and Negative 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	≤ 0.100 (GpIV row)	≥ 0.900 (GPIV row)

INTERPRETATION OF TEST RESULTS

Test results showing OD values equal to or greater than 2X the value obtained for the negative control platelet 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 or of the test platelets, exposure of substrate to stray light, omission of test reagents, exposure to higher or lower than prescribed temperature requirements, insufficient or excessive platelets, incompatible ABO groups, or omission of steps.

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, or antibodies to platelet glycoproteins other than those on Ia/IIa, Ib/IX, and GPIV.

In vivo sensitized platelets have not been tested using this product.

SPECIFIC PERFORMANCE CHARACTERISTICS

When properly stored and used according to the procedures described above, this product can detect IgG antibodies reactive with epitopes on GPIb/IX, GPIa/IIa, and GPIV glycoproteins.

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

Performance Evaluation

Comparative Method

MACE ^{®2}		Positive	Negative	Total
	Positive	8	6	14
	Negative	1	132	133
	Total	9	138	147

Agreement: 95.2%

Co-positivity: 88.9% Co-negativity: 95.6%

Comparative Method: GTI-PAK^{®2}

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20925 Crossroads Circle, Suite 200
Waukesha, WI 53186-4054 USA
(262) 754-1000 OR 1-800-233-1843

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Qarad b.v.b.a.
Volmolenheide 13
B-2400 Mol
Belgium



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