

# Instructions for use



Sanquin Reagents B.V.  
Plesmanlaan 125  
1066 CX Amsterdam  
The Netherlands

Phone: +31 20 5123599  
Fax: +31 20 5123570  
Reagents@sanquin.nl  
www.sanquin.org/reagents

## MASPAT kit

REF K1360

IVD C€

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For professional use only

Solid phase assay for the detection of platelet antibodies

### General information

The MASPAT kit (Monoclonal Antibody Solid-phase Platelet Antibody Test) contains coated MASPAT microplates (12 strips of 8 wells), MASPAT LISS, MASPAT anti-IgG, MASPAT positive control and MASPAT negative control. The MASPAT kit meets the requirements of the concerned standards and guidelines. Performance characteristics are mentioned in the release document, which is supplied with the product upon request. The principle of the test is the solid phase technique, which is based on antigen/antibody reaction. A monolayer of donor platelets is immobilized by centrifugation onto the surface of the microplate wells coated with platelet-specific mouse monoclonal antibody. Patient serum and LISS (Low Ionic Strength Solution) are incubated in the appropriate wells, allowing serum antibodies to bind to the immobilised platelet monolayer. After incubation, non-bound serum components are removed by washing. Platelet-bound antibodies are detected by the addition of mouse monoclonal anti-human IgG and human IgG-sensitized erythrocytes (MASPAT Indicator Red Cells) and subsequent centrifugation of the microplate. In case of a positive reaction the anti-human IgG and MASPAT Indicator Red Cells bind to the IgG-antibodies on the platelet monolayer. Positive reactions are thus characterised by adherence of MASPAT Indicator Red Cells throughout the surface of the wells, whereas negative reactions produce discrete pellets of MASPAT Indicator Red Cells in the middle of the well. The inclusion of MASPAT positive control and MASPAT negative control with each series of tests is strongly recommended.

If human platelet typing reagents are available MASPAT can be used for typing platelet specific antigens on donor and patient platelets. MASPAT can also be used to determine platelet specific antibodies when typed donor platelets are used.

### Kit parts:

**MASPAT microplate** (REF K1366): an U-bottom microplate consisting of 12 strips of 8 wells. All the wells are coated with platelet-specific monoclonal antibodies (clone: tromb7 (6G9)). Store at 2–8°C. The MASPAT microplate is vacuum sealed in a plastic pouch containing desiccant. After removal from the pouch the wells may be used for a period of up to 4 weeks if stored at 2–8°C.

**MASPAT LISS 10 ml** (REF K1362): Low Ionic Strength Solution. Store at 2–8°C.

**MASPAT positive control 1 ml** (REF K1363): contains platelet-specific antibodies. Store at 2–8°C.

**MASPAT negative control 1 ml** (REF K1364): contains no platelet-specific antibodies. Store at 2–8°C.

**MASPAT anti-IgG 6 ml** (REF K1361): mouse monoclonal antibody (clone: MH-165) specific for human IgG (IgG1, IgG2, IgG3). Store at 2–8°C.

### Precautions

For in vitro diagnostic use only. Reagents should be stored at 2–8°C. Leaking or damaged vials may not be used. Reagents (unopened or opened) should not be used beyond the expiration date, which is printed on the label of the vial. NaN<sub>3</sub> 0.1% (w/v) is used as preservative. Although all blood products are tested for infectious diseases and found negative, the reagents cannot be assumed to be free from infectious agents.

Care must be taken in the use and disposal of each container and its contents. Turbidity may indicate microbial contamination. To recognise reagent deterioration, testing of the reagent as part of the laboratory quality control program using appropriate controls is recommended. Waste-disposal, after completion of the test, should be performed according to your laboratory regulations.

### Specimen collection and preparation

**Serum samples** should be obtained from clotted blood following centrifugation at 1500 rcf for 10 minutes. If testing of the serum is delayed, storage should be at <–18°C.

Preparation of the specimen is described in the respective test procedures.

Platelet Concentrates (PC) or Platelet Rich Plasma (PRP) can be used in the test. For preparation of PRP, whole blood collected in EDTA is centrifuged at 400 rcf for 10 minutes. The PRP-fraction can be used directly, undiluted.

### Test procedure

Materials required but not supplied:

- MASPAT Indicator Red Cells 0.3% (REF K1139) of sensitized erythrocytes in preservation medium.
- Multichannel pipette suitable for 50, 100 and 150 µl and disposable tips.
- Phosphate Buffered Saline (PBS) and Tween 20.
- Reservoirs for multichannel pipette.
- Disposable PLASTIC transfer pipettes.
- Centrifuge suitable for microplates.
- 37°C incubator
- Microplate shaker.

Allow all reagents to reach room temperature (18–25°C).

1. Remove the microplate with the required number of microplate wells from the pouch. The unused wells can be stored in the plastic pouch with the desiccant.
2. Add 1 drop (50 µl) of donor PRP or PC with a **plastic** transfer pipette to the wells needed (for patient serum, MASPAT POSITIVE CONTROL, MASPAT NEGATIVE CONTROL). It is advised to perform the MASPAT POSITIVE- and MASPAT NEGATIVE CONTROL for each strip of 8 wells.
3. Centrifuge the microplate for 5 minutes at 50 rcf to immobilize the platelets onto the surface of the microplate wells (**without braking**).
4. Decant and remove any unbound platelets by washing the microplate wells manually 3\* times with 150 µl of PBS/Tween 0.005%. The wash solution should be added dropwise using a multichannel pipette. Decant PBS/Tween between washes by inverting and flicking.
5. Add 2 drops (100 µl) MASPAT LISS to each well. The LISS is purple in color.
6. Add 1 drop (50 µl) of MASPAT POSITIVE- or NEGATIVE CONTROL to the appropriate wells. The positive control checks if an adequate platelet monolayer has been obtained from the donor.
7. Add 1 drop (50 µl) of patient serum to the remaining wells containing donor platelet monolayers.
8. Shake on a microplate shaker for 10 seconds at 800 rpm (Sarstedt, TPM-2). The purple color of the LISS should change in blue.
9. Incubate the microplate at 37°C for 30 minutes (seal the microplate during incubation).
10. Decant and wash the microplate wells manually 5\* times with 150 µl of PBS/Tween as in step 4.
11. Add 1 drop (50 µl) of MASPAT anti-IgG reagent to each well immediately after washing.
12. Add 1 drop (50 µl) of MASPAT INDICATOR RED CELLS to each well. Shake gently.
13. Centrifuge the microplate at 200 rcf for 5 minutes (**without braking**).
14. The reactions can now be read either macroscopically or using an automatic reader.  
\* If the washing is done automatically it is advised to validate the washing steps.

### Interpretation

A positive or weak positive reaction (erythrocytes form a layer over the bottom of the well) indicates the presence of platelet and/or HLA specific antibodies in the serum.

A negative reaction (erythrocytes form a button in the bottom of the well) indicates the absence of platelet and/or HLA specific antibodies in the serum.



positive



weak positive



negative

The MASPAT negative control has to be performed to determine auto antibodies bound to the donor platelets.

If a patient auto control is required the patient serum has to be tested against the patients own platelets derived from an EDTA blood sample.

Enhancing the reactivity of platelets: by freezing the donor platelets (PC or PRP) for minimal 30 minutes (<–18°C) up to 8 weeks their reactivity will be enhanced.

PC's can be diluted with sequestrine buffer.

Composition of stock solution sequestrine buffer (10x concentrated):

- 0.175 M Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O
- 0.089 M Na<sub>2</sub>EDTA·2H<sub>2</sub>O (Titriplex III)
- 1.54 M NaCl

Dissolved in demi water

pH: 6.8–7.0

Sequestrine buffer 10x concentrated should be diluted 1:10 in demi water before use.

Store at room temperature (18–25°C). Shelf life stock solution: 1 year; working solution 1 week.

### Limitations

The wash solution should be added slowly, as vigorous washing may remove both bound and unbound platelets. The surface of the well should be opaque if the wash procedure has been carried out properly. Irregular "holes" in the platelet monolayer indicate an incorrect washing procedure. If this occurs a new monolayer should be made, using new microplate wells (steps 3–5).

False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.

False positive results may occur if the donor / patient platelets are ABO incompatible with the serum being tested, if IgG auto antibodies are bound to the donor platelets, or if the concentration of platelets is too low (<0.5 x 10<sup>9</sup>/ml). To test if the donor platelets carry auto antibodies, one can perform the test with the donor platelets + negative control or with the donor platelets + donor serum.

False negative results may occur with the MASPAT positive control when the used platelets lack the antigens HPA-1a and HPA-3a.

If the centrifugation speed and time have not been optimized poor platelets adherence to the microplate may occur. Suboptimal coating of the wells will result in an inadequate test performance by reducing both the sensitivity and the strength of reactions in the final test results.

Insufficient washing prior to the addition of MASPAT anti-IgG reagent and/or MASPAT Indicator Red Cells may result in false negative reactions.

Undercentrifugation of the microplate after the addition of MASPAT Indicator Red Cells can lead to false positive results in all tests (including negative control). False positive reactions may also be a result from failing to correctly resuspend the MASPAT Indicator Red Cells.

Overcentrifugation of the microplate after the addition of MASPAT Indicator Red Cells can lead to false negative results in all tests (including positive control).

**References**

Lown, J.A., Ivey, J.G., Evaluation of a solid phase red cell adherence technique for platelet antibody screening. Department of Haematology, Royal Perth Hospital, Western Australia. (Transfus. Med. 1991).

*Sanquin products are guaranteed to perform as described in the original manufacturer's instructions for use. Strict adherence to the procedures, test layouts and recommended reagents and equipment is essential. Sanquin declines all responsibility arising from any deviation thereof.*