

Previous MAIPA Protocol

MAIPA Assay

This consensus protocol was compiled from the methods used by the participants of the Platelet Serology Exercises organised by NIBSC. More weight was given to the protocols from laboratories with consistently high performance in the exercises, however, it may not perform any better than the original published method (Kiefel *et al*, Blood 1987; 70, 1722-1726).

The protocol has been used successfully at NIBSC but it should be validated locally.

Reagents

Coating Buffer

1.59 g Na_2CO_3 , 2.93 g NaHCO_3 , 0.2 g NaN_3 , add dH_2O to 1 litre, adjust pH to 9.6

TBS Wash Buffer

3.03 g Tris, 22 g NaCl, 12.5 mL Nonidet P40, 1.25 mL Tween²⁰, 1.25 mL 1M CaCl_2 , add dH_2O to 2.5 litres

Solubilisation Buffer

1.21 g Tris, dissolve in 950 ml isotonic saline, pH 7.4. Add 5 mL Nonidet P40, add isotonic saline to 1 litre

Goat Anti-Mouse IgG

Jackson ImmunoResearch, Code 115-005-071. Dilute to 3 ug/mL in coating buffer. Do not keep any un-used diluted reagent.

Peroxidase Conjugated Goat Anti-Human IgG

Jackson ImmunoResearch, Code 109-035-008. Dilute to 1 in 5000 - 1 in 30,000 (determined by previous experiment) in TBS wash buffer. Do not keep any un-used diluted reagent.

Substrate Solution

Dissolve four 3.5 mg tablets 1,2 phenylenediamine dihydrochloride (OPD. 2HCl) (Dako code S 2045) in 12 ml distilled water in foil-covered container. Add 5ul 30% H₂O₂ immediately before use. Do not keep any un-used diluted reagent.

Stop Solution

Add 28 ml 95-97% H₂SO₄ to 972 ml distilled water. **NB Do not mix the water into concentrated acid**

MAIPA Assay (microplate method)

A. Preparation of Coated F-Well Microplate

1. Prepare goat anti-mouse IgG as above and aliquot 100uL/well into 2 Flat-well microplates (NUNC 44204A) for duplicate testing.
2. Incubate microplates at 4°C overnight (or at least two hours at room temperature)
3. Store sealed coated plates for up to 2 weeks at 4°C.
4. 30 mins before plates are required on day of testing, wash four times with TBS wash buffer.
5. Leave last wash in wells for 30 min at 4°C to block plate and prevent non-specific binding at subsequent stages. Decant buffer before use.

B. Preparation of platelets

1. Take blood from group O donors into EDTA or citrate.
2. Centrifuge at 500g for 10 min in bench-top centrifuge
3. Remove the top 3/4 of the PRP from the top of the tube and transfer to 10ml conical centrifuge tube.
4. Make up to 10mL with PBS/EDTA buffer. Centrifuge at 2,000g approx for 5 mins and decant supernatant.
5. Re-suspend cells gently in 2mL buffer and repeat step 4. twice more.
6. Re-suspend platelets in PBS/EDTA at 200x 10⁹/L approx. Store at 4°C for up to 2 weeks.

C. First incubation

1. For each sample to be tested (no. of tests plus controls x number of donors x no. of mabs) aliquot 100uL platelet suspension into U-well microplate and spin at 900g in a benchtop microplate centrifuge for 3 min. Include 1 negative control and 1 weak positive control which has been calibrated against the National anti-HPA-1a standard (NIBSC code 93/710)
2. Decant supernatant and resuspend platelet pellets in 50uL test serum or plasma and incubate at 37°C for 30 min.
3. Wash platelets by adding 200 uL PBS/EDTA to each test and centrifuge as in C.1 above. Re-suspend platelet pellet by using microplate shaker and wash once more.

D. Incubation with mab

1. Re-suspend platelet pellets in 40uL diluted mouse monoclonal antibody (make sure cells are fully re-suspended using microplate shaker or pipette) and incubate for 30 mins at 37°C.
2. Wash platelets three times.

E. Lysis

1. Lyse platelets by re-suspending each pellet in 130uL solubilisation buffer. Resuspend cell pellet using a pipette not the microplate shaker, to avoid carry over. Incubate for 30 mins at 4°C.
2. Block coated F-well microplate, see sections A.4 & A.5 above.
3. Centrifuge microplate at maximum speed in bench top centrifuge (1400g approx.) for 15 mins at room temperature.
4. For each test aliquot 130uL TBS wash buffer into U-well microplate.
5. Remove 100uL from each supernatant from step E.3 and dilute in 130uL TBS wash buffer from step E.4.

F. Attachment to solid phase

1. Transfer 100uL diluted platelet lysate, in duplicate, to pre-coated and blocked F-well microplate (from step E.2 above). Add 100uL TBS wash buffer to 4-8 pre-coated wells on each plate (= reagent blank). Incubate at 4°C for 90 min or overnight.
2. Wash microplate wells 4 times with 200uL TBS wash buffer.

G. Anti-IgG incubation

1. Add 100uL diluted peroxidase labelled anti-human IgG to each well and incubate at 4°C for 90 min.
2. Wash microplate wells 6 times with 200uL TBS wash buffer. Blot top face of microplate dry in-between washes by placing on absorbent tissue.

H. Colour development

1. Add 100uL substrate solution to each well and leave plate at room temperature.
2. Stop the reaction by adding 100uL 0.5 M H₂SO₄ when a suitable colour intensity has developed. Read the absorbance at 490nm (for dual wavelength ELISA readers a reference filter between 630 and 650 is suitable) within 1 hour.

N.B. 2mL microfuge tubes can be used instead of U-well microplates, in which case the centrifugation at part E step 3. can be increased to >10,000g for 20 min at 4°C.