BAMOMAB

Protocol MICA-Sandwich ELISA

1. COATING

- Incubate capture anti-MICA mAb (AMO1) at 5µg/ml (dilute stock solution 1:200 in PBS) overnight at 4°C in an ELISA plate (100µl/well).
  - BAMOMAB recommends *Nunc-Immuno 96 MicroWell Plates/MaxiSorp* (from Nunc, Cat-Nr. 442404).

2. BLOCKING

- Add 15% BSA-PBS (100µl/well) to AMO1 and incubate for 1h at 37°C.

- After 1h incubation: Wash plate 4x with TWPBS (0.05%TWEEN-20 in PBS).

3. SAMPLING

- Add samples/standard diluted in 7.5% BSA-PBS (100µl/well) for 2h at 37°C.
  - Standard: Use rMICA*04 at 20 ng/ml (dilute stock 1:5 in 7.5% BSA-PBS and titrate (in 1:2 dilutions) in 12 steps to 10pg/ml).
  - Serum samples: Dilute 1:3 (1Vol. Serum + 2 Vol. 7.5% BSA-PBS) and spin 15 min with max. rcf in desk centrifuge. Take supernatant for analysis.

- After 2h sample incubation: Wash plate 4x with TWPS.

4. SANDWICHING

- Add purified anti-MICA/B mAb BAMO3 at 1µg/ml (dilute stock solution 1:200 in 7.5% BSA-PBS) (100µl/well) and incubate for 2h 37°C.

- After 2h BAMO3 incubation: Wash plate 4x with TWPS.

5. DETECTION

- Add HRP-conjugated anti-mouse IgG2a Ab (dilute 1:10.000 in 3.75% BSA-PBS) (100µl/well) for 1h at 37°C.
  - BAMOMAB recommends *HRP-conjugated goat anti-mouse IgG2a* (from Southern Biotechnologies Cat. Nr. 1080-05).

- After 1h incubation: Wash plate **6x** with 0.05%TWPS.

6. SUBSTRATE

- Develop with HRP substrate
  - BAMOMAB recommends *TMB 2-component microwell peroxidase substrate kit* (from KPL, Cat. Nr. 50-76-00).

  (mix equal volumes of TMB Peroxidase Substrate and Peroxidase Sol. B and add 100µl of the mixture/well. Incubate at RT 5-60 min)

7. STOP AND MEASURE

- Add 100µl 1M phosphoric acid and read results at 450 nm wavelength.

Attention: Do not use AZIDE-containing solutions after step 4. SANDWICHING !