Detecting MPO:DNA complexes as a marker of NETosis, Dr. Lood Protocol

1. Coat a 96-well high-protein binding ELISA plate with 100 uL anti-MPO antibodies (5 ug/mL in PBS, Biorad) at 4 C o/n.
2. Wash x3 with ELISA wash buffer.
3. Add 200 uL blocking buffer (1% BSA in PBS) for 2 hrs at RT with shake.
4. Wash x3 with ELISA wash buffer.
5. Add 100 uL samples (10% plasma/serum/purified NETs), diluted in 1% BSA in PBS with 2 mM EDTA, and incubate at 4 C o/n.
6. Wash x3 with ELISA wash buffer.
7. Add 100 uL detection antibody (anti-DNA-HRP, part of the Cell Death Detection Kit, Roche), diluted 1/100 and incubate for 2 hrs RT with shake.
8. Wash x3.
9. Add 100 uL substrate (TMB 1:1 of the two components). The samples will turn blue.
10. Add 2 N sulfuric acid to stop the reaction - the samples will turn yellow.
11. Analyze absorbance (450 nm) using plate reader.